Thunder Bay Area of Concern Beneficial Use Impairment Assessment Report

Bird or Animal Deformities or Reproduction Problems

Welcome Islands, Thunder Bay
Environment and Climate Change Canada

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Summary

The attached technical report 'Assessment of the Wildlife Reproduction and Deformities Beneficial Use Impairment in the Thunder Bay Area of Concern' details the methods, field and laboratory analyses, results, discussion and conclusions of a three year study undertaken in 2012, 2014 and 2015 by researchers within the Science and Technology Branch of Environment and Climate Change Canada (ECCC). The study assessed the status of the Bird or Animal Deformities or Reproduction Problems beneficial use impairment (BUI) in the Thunder Bay Area of Concern (AOC) by examining herring gull and double-crested cormorant colonies. Fish-eating wildlife such as herring gulls and double-crested cormorants are important indicators of exposure to persistent contaminants in the aquatic environment. Results of the three-year study clearly demonstrate that this BUI is not impaired within the Thunder Bay AOC.

When Thunder Bay was identified as an AOC in 1987, discharges of pollutants from local pulp and paper industries and wastewater treatment plants, as well as atmospheric deposition and urban runoff, had impaired water quality and ecosystem health. At the time, contaminants of concern included dioxins and furans, mercury and polychlorinated biphenyls (PCBs), which the Stage 1 Remedial Action Plan report (1991) identified as a potential risk for causing deformities and reproduction problems in birds and animals. Studies were planned to determine if deformities and reproduction problems were occurring because of chemical contamination, and the status of this BUI was identified as 'requires further assessment'.

In the years following the Stage 1 Remedial Action Plan, no bird or animal deformities were reported within the AOC and the BUI status was changed to 'not impaired' in the Stage 2 Remedial Action Plan (2004), although the results of an ECCC study initiated in 2000 were not yet available.

From 2000-2008, ECCC conducted various assessments of reproduction, health effects and contaminant levels in colonial waterbirds nesting within the Thunder Bay AOC. While contaminant levels in herring gull eggs were similar to reference colonies and reproduction levels and health effects were largely comparable, there was some evidence that clutch volume and egg size differed in herring gulls nesting on Mutton Island in the AOC compared to those nesting at the non-AOC Lake Superior reference colony at Granite Island. This resulted in the status changing back to 'requires further assessment' in 2010.

To conclusively determine the status of this beneficial use, ECCC undertook a three-year study in 2012, 2014 and 2015 to assess colonial waterbird health in the Thunder Bay AOC. The results of the study demonstrate that there are no bird deformities or reproduction problems due to concentrations of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) or other organochlorine compounds, dioxins and furans, or mercury in the Thunder Bay AOC.
In brief, some of the findings from the three-year study that led to the recommendation that this BUI is not impaired within the Thunder Bay AOC include:

i) Embryonic viability: Artificial incubation studies conducted in the lab revealed that embryonic viability was similar for fertilized embryos collected within the Thunder Bay AOC and from reference colonies. Embryonic development is a sensitive stage in terms of potential pollutant impacts and the lack of impact on this endpoint indicates no impairment from contaminants.

ii) Productivity: This is a measure of fledging success/recruitment. In the Thunder Bay AOC, a stable herring gull population is being maintained (based on number of fledglings per nest), with no evidence of impairment from contaminants.

iii) Incidence of deformities: Over the three-year study, no deformities were observed among herring gull embryos incubated in the lab or herring gull chicks assessed on the breeding colonies within the Thunder Bay AOC.

iv) Contaminant burdens: Levels of contaminants such as PCBs, dioxins and mercury in herring gull embryos and double-crested cormorant eggs from the Thunder Bay AOC were comparable to, or lower than, burdens in embryos or eggs from reference colonies.

v) Other biochemical endpoints: No other adverse health effects relating to growth and development due to contaminants were found in herring gull chicks.

Assessment of the Wildlife Reproduction and Deformities Beneficial Use Impairment in the Thunder Bay Area of Concern

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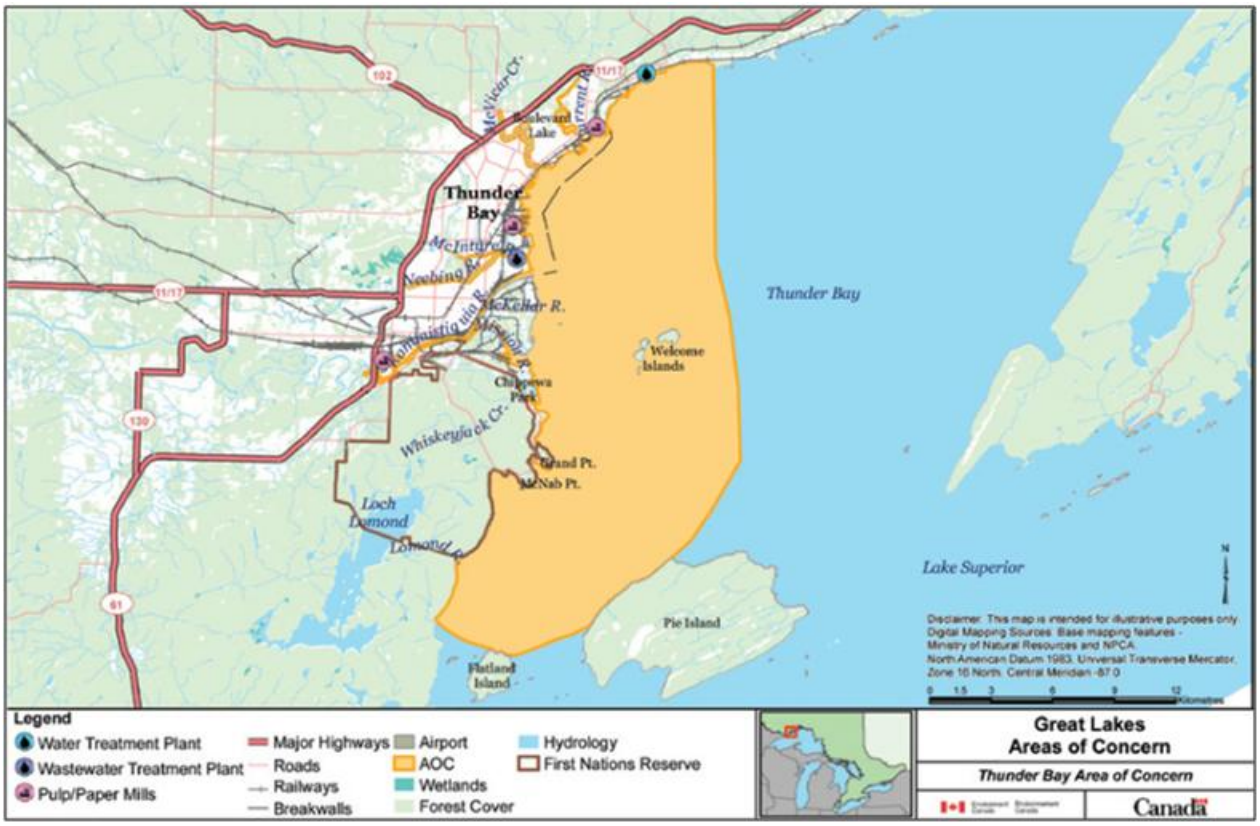
ABSTRACT

Reproduction and development were examined in herring gulls (*Larus argentatus*) breeding within the Thunder Bay Area of Concern in 2012, 2014 and 2015. Freshly-laid eggs were collected from two colonies within the Area of Concern (AOC) and a reference colony outside of the AOC, artificially incubated in the laboratory and assessed for embryonic viability, incidence of embryonic deformities, contaminant burdens and other biochemical endpoints (i.e., stable isotopes). Productivity was determined at the colonies when chicks were ≥ 21 days old and chicks were examined for morphological deformities as well as other biological endpoints. A second aquatic-feeding colonial waterbird species, the double-crested cormorant (*Phalacrocorax auritus*), nesting at a colony within the AOC was also selected for assessment of contaminant burdens. Overall, embryonic viability of herring gulls was high at AOC colonies and herring gull productivity at these colonies was within the range required to maintain a stable population. No embryonic deformities were evident in gull eggs incubated in the laboratory and no morphological deformities were found in ≥ 21-day-old herring gull chicks from AOC colonies (based on sample sizes of 10-33 chicks). Importantly, contaminant burdens (e.g., PCBs, 2,3,7,8-TCDD, and mercury) in gull embryos and double-crested cormorant eggs from the Thunder Bay AOC were not notably elevated and were comparable to or lower than burdens in embryos or eggs from respective reference colonies in these years. No adverse health effects were evident in gull chicks based on measurements of biochemical endpoints relating to growth and development and immune function. Population trends based on nest count surveys indicate greater numbers of nests for both species at AOC study colonies in 2007 and 2012 compared to earlier decades. Lake-wide population trends based on decadal surveys conducted on the Canadian and American sides of Lake Superior indicate that nest numbers of herring gulls have fluctuated while cormorant nest numbers have increased steadily since the late 1970s. Large declines in concentrations of sum PCBs and other organochlorines, dioxins and TEQs in herring gull tissues since the early 1990s indicate that exposure to these compounds has decreased in herring gulls foraging in the AOC. While no change in mercury levels was found in herring gulls in the AOC, this pattern was similar to that found at other Lake Superior herring gull colonies during this period. In summary, concentrations of PCBs, other organochlorine compounds, PBDEs, dioxins and furans, and mercury were not sufficiently elevated to adversely impact the reproductive success and development of herring gulls and cormorants nesting in the Thunder Bay AOC.

INTRODUCTION

The Thunder Bay Area of Concern (AOC) is one of 43 Great Lakes AOCs that were initially identified by Canada, the United States and the International Joint Commission (IJC) as specific locations where local environmental degradation had severely impacted the area's ability to support aquatic life. The border of the AOC extends approximately 28 kilometres along the shoreline of Lake Superior from north of Bare Point to Flatland Island and up to 9 kilometres offshore, including the Welcome Islands (Figure 1). Historical discharges of pollutants from local pulp and paper industries and wastewater treatment plants, as well as atmospheric deposition and urban runoff impaired water quality and contaminated sediment in the Bay (Thunder Bay RAP Team 1991). Contaminants of concern included dioxins and furans, mercury, and polychlorinated biphenyls (PCBs) which contributed to exceedances of water

Figure 1. The Thunder Bay Area of Concern.



quality objectives, sediment quality guidelines and/or fish consumption guidelines (Thunder Bay RAP Team 1991, 2004).

Fourteen beneficial use impairments (BUIs) were used by the Canadian and U.S. federal governments to identify and assess the extent of environmental degradation and direct restoration and remedial activities. One of these BUIs, "bird or animal deformities or reproduction problems", addresses contaminant exposure or other anthropogenic environmental stressors on reproductive success or deformity rates in wildlife. Initially, this BUI was designated as "requires further assessment" since no formal studies had been conducted (Thunder Bay RAP Team 1991). The status of the BUI was subsequently changed to "not impaired" following no reports of bird and animal deformities in the AOC and observations that cormorant populations were reproducing at normal levels and numbers of colonies on Lake Superior had increased since the early 1970s (Weseloh *et al.* 1995; Thunder Bay RAP Team 2004). However pending the results of additional studies conducted by the Canadian Wildlife Service (CWS) initiated in 2000, the status of this BUI was changed back to "requires further assessment" (Environment Canada and the Ontario Ministry of the Environment 2011). In 2012, studies of the potential effects of contaminants on reproduction and development in aquatic-feeding wildlife were initiated by Environment and Climate Change Canada (ECCC) to more fully evaluate and assess the status of this BUI in the Thunder Bay AOC. The approach used here has been implemented in the St. Marys River (Ontario) and Hamilton Harbour AOCs for similar assessments of this BUI.

Fish-eating wildlife, such as colonial waterbirds, are important indicators of exposure to persistent contaminants in the aquatic environment (Fox and Weseloh 1987). As top predators, they occupy a high trophic level in the aquatic food web and therefore can accumulate high levels of contaminants, which may in turn adversely affect their reproductive health and development. Two colonial waterbird species, that breed and forage within the Thunder Bay AOC, were selected for assessment purposes. The herring gull (*Larus argentatus*) is a long-lived, primarily fish-eating colonial waterbird that from the time it reaches breeding age is a year-round resident in the Great Lakes basin. A second aquatic-feeding colonial waterbird species, the double-crested cormorant (*Phalacrocorax auritus*), was selected for assessment of contaminant burdens. This species feeds almost exclusively on fish compared to herring gulls, which are opportunistic feeders that will consume terrestrial prey if fish are not readily available. This close connection to the aquatic environment is vital for assessment of local conditions in the AOC.

In 2012, 2014 and 2015, breeding colonies of herring gulls were studied by ECCC in the Thunder Bay AOC. Freshly-laid eggs were collected for artificial incubation in the laboratory to assess embryonic viability, incidence of embryonic deformities, contaminant burdens and biochemical endpoints (i.e., stable isotopes). Under controlled laboratory conditions, this method assesses the effects of embryonic exposure to potentially high levels of contaminants during critical periods of development. Reproduction and development were also assessed in wild populations with visits to colonies to monitor productivity and examine chicks for morphological deformities and to measure additional biochemical endpoints (i.e., stress hormone, thyroxine levels) relating to growth and development as well as immune function, which could be affected by increased contaminant exposure. The results of this study will be used to assess the status of the wildlife reproduction and deformities BUI in this AOC. The results of earlier studies conducted by CWS and initiated in 2000 in the AOC are referenced in the discussion with further details provided in the Supplementary Information section.

METHODS

Two herring gull colonies were selected within the Thunder Bay AOC at Mutton Island (48°22’15”N, 89°11’43”W) and an unnamed island (48°21’0”N, 89°08’34”W) located further out from the City of Thunder Bay shoreline and south of the two main Welcome Islands (Figure 2). Specifically, there are two small, unnamed islands situated approximately 0.4 kilometers and 0.6 kilometers south of the Welcome Islands. The AOC colony for this study is the latter island that is also smaller in size (0.34 hectares). For our purposes, this study colony will be hereafter referred to as “Welcome Islands-a”. Historically, colonial waterbird studies have also been conducted on the larger of these two unnamed islands (0.98 hectares) and this site will be subsequently referred to as “Welcome Islands-b” (48°21’07”N, 89°08’35”W). Initially Granite Island (48°43’11”N, 88°27’33”W), located approximately 65 km northeast of the AOC in Black Bay of Lake Superior, was selected as the AOC reference colony. However, during the first visit in late April of 2012, this portion of the lake was not yet ice-free and gulls had not begun laying eggs. Since it was not possible to collect eggs for artificial incubation or to build enclosures for the field study at Granite Island at that time, Double Island (46°10’24”N, 82°51’50”W) in the North Channel of Lake Huron was selected as the alternate reference site for assessment of these components. This coincided with work that was underway for a similar study in the St. Marys River AOC (Ontario) where Double Island served as the non-AOC reference site.

Figure 2. Colony locations for herring gulls and double-crested cormorants in the Thunder Bay AOC in 2012, 2014, and 2015.

Visits to each colony were made at two times during the breeding season for gulls: 1) egg laying (late April) and 2) when chicks were $\geq$ 21 days old (mid-May) in 2012 and 2014 to assess reproduction and various parameters of health. In 2014, due to late spring and high water levels, productivity could not be sufficiently determined at Welcome Islands-a because 10 of the 12 nest enclosures were washed out. As a result, an additional year of field study (2015) was required to estimate productivity at AOC colonies. Health parameters were also examined in 2015 to supplement the two earlier years of data. During the first visit, 15 freshly-laid eggs (i.e., not incubated) were collected from one-egg nests at each colony for artificial incubation in the laboratory at the National Wildlife Research Centre (NWRC) in Ottawa. Embryonic viability, incidence of embryonic deformities, contaminant burdens, and stable isotope signatures were determined. In addition, a thorough nest count was conducted and contents were recorded at the colony. Individual nest enclosures (~1m in diameter and 16" high) were constructed around twelve 3-egg nests at each colony. As a measure of colony health, egg measurements for up to forty-two 3-egg clutches were recorded (in millimetres) and egg volume calculated as:

$$\text{Egg volume } (cm^3) = 0.489 \times (\text{length} \times \text{breadth}^2)/1000$$

Total clutch volume was determined as the sum volume of the three eggs in the clutch. Intraclutch variation in egg size was calculated as the difference in volume between the largest and smallest egg in the clutch divided by the largest egg size (i.e., volume) and multiplied by 100.

During the second visit, when chicks from enclosed nests were $\geq$ 21 days old, productivity was calculated as:

$$\text{Productivity} = \text{no. of} \geq \text{21-day-old chicks/no. of enclosed nests}$$

Enclosed nests that had been abandoned, damaged by high water levels, or where there was evidence that chicks had escaped were not included in estimates of productivity. Body measurements of chicks, including mass, tarsus, wing cord, and culmen length were recorded and chicks were banded with a stainless steel USFW band. Chicks were examined for morphological deformities when they were $\geq$ 21 days old. Health effects were also assessed in chicks at this stage. A blood sample was collected from the brachial vein of chicks using a 25 gauge x 5/8" needle and heparinized syringe to examine thyroxine concentrations in plasma (see below for further details). In addition, two secondary covert feathers were collected to quantify corticosterone concentrations as a measure of stress over time in herring gull chicks. Immune status of chicks was evaluated using a phytohemmagglutinin (PHA) skin test.

Double-crested cormorant eggs were also collected for assessments of contaminant burdens. Cormorants nesting on Welcome Islands-a served as the AOC colony and Black Rock (46°06'59"N, 82°50'00"W) in the North Channel served as the corresponding reference colony. In May of 2012 and 2014, 10 or 13 eggs were collected from nests containing $\geq$ 3 eggs at each colony. After collection, the eggs were sent to NWRC where contents were placed in chemically-cleaned glass jars, homogenized, and frozen until chemical analysis. Eggs from each colony were pooled together as a single sample for chemical analysis.

Artificial Incubation of Eggs:

In 2012 and 2014, unincubated herring gull eggs were collected in the field from nests containing a single egg, transported to NWRC in insulated coolers with foam inserts and set in a Petersime incubator (model# MX-1) at 37°C, 58% humidity and turned every two hours. It was not possible to follow this protocol at Double Island in 2014 due to late ice-out conditions and the inability to access the colony during the early-laying period. Consequently, subsequent collection of eggs and transport to the laboratory at this later egg-laying period likely compromised embryonic viability of eggs under artificial incubation conditions. Following this, the results of the artificial incubation study conducted at Double Island in 2013 were used for comparisons with the AOC colonies in 2014. The incubated eggs that were collected from Double Island in 2014 however, were analyzed for contaminant burdens (see details below).

Just prior to the pipping stage of development (i.e., embryonic day 26-27), embryos were removed from their shells and euthanized by decapitation. Each embryo was examined for physical deformities. Embryonic viability was determined as the number of viable embryos that survived to the designated embryonic day (i.e., just prior to pipping) divided by the total number of fertile eggs. Eggs that were nonviable were staged if possible (e.g., infertile; early, mid or late embryo death). Egg contents, including yolk sac, whole carcass, and shell membranes, were collected in chemically-cleaned glass jars, homogenized, and frozen until chemical analysis for contaminants. Ten to fifteen embryos were randomly selected from each colony for chemical analysis in the two study years.

Contaminant Analyses:

Chemical analyses of herring gull embryos and cormorant eggs for organochlorine compounds and polybrominated diphenyl ethers (PBDEs) were conducted at the Great Lakes Institute for Environmental Research at the University of Windsor (2012-AOC colonies) and at NWRC (2012-reference colonies,

2014-AOC and reference colonies). Organochlorine compounds measured included *p,p'*-DDE (dichlorodiphenyldichloroethylene), oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene (HCB), dieldrin, heptachlor epoxide (HE), mirex, octachlorostyrene (OCS), and polychlorinated biphenyls (PCBs). Sum chlordane is based on the sum concentrations of oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor. Prior to chemical analysis, thawed embryos and eggs were homogenized and then underwent neutral extraction and removal of lipids and biogenic compounds by gel permeation chromatography and further clean up by Florisil column chromatography. Quantitative analysis of organochlorine compounds was performed using capillary gas chromatography coupled with a mass selective detector (GC-MSD) operated in selected ion monitoring mode. PBDEs were quantified by gas chromatography high resolution mass spectrometry methods using a time-of-flight mass spectrometer (GC-MS-TOF) at GLIER and by GC-MSD operated in the NICI mode at NWRC. Sum PCBs were based on the sum concentrations of 35-62 individual or co-eluting PCB congeners found above the limit of detection depending on the year and laboratory performing the analysis. Similarly, sum PBDEs were based on the sum concentrations of 15 or 25 individual or co-eluting PBDE congeners found above the limit of detection. Certified internal standards were used for quantification and certified reference materials, blanks and duplicate samples were analyzed for quality assurance purposes. Concentrations of organochlorines and PBDEs are reported in µg/g on a wet weight basis. Detection limits for organochlorine compounds and individual PBDE congeners ranged from 0.00002-0.0003 µg/g.

Embryos and eggs were analyzed for non-*ortho s*ubstituted PCBs, polychlorinated dibenzo-*p*-dioxins, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and polychlorinated dibenzofurans using gas chromatography-high resolution mass spectrometry (GC/HRMS) at RPC Laboratory in Fredericton, New Brunswick. Methods were based on US EPA Method 1613B and 8290A for dioxins and furans and on US EPA Method 1668C for non-*ortho* PCBs. Reference materials, blanks, and duplicates were analyzed for quality assurance purposes. Samples were analyzed as a single pool consisting of 10-15 gull embryos at each colony. Embryos from Double Island in 2012 were not analyzed for these compounds since the results of analysis from the year prior (2011) were available when eggs were also collected as part of the St. Marys River AOC study and artificially incubated in the lab under identical conditions (single pool of five embryos). Double-crested cormorant eggs were analyzed for non-*ortho* PCBs, dioxins and furans in 2014 only and as a single pool comprising 10 eggs from each colony.

Total mercury was quantified on a dry weight basis using an Advanced Mercury Analyzer (AMA-254) as described in CWS Method No. MET-CHEM-AA-03I at NWRC. Certified reference materials and duplicate samples were also analyzed to ensure correct calibration, accuracy, and reproducibility of test methods. Mercury concentrations in 2012 and 2014 embryos and eggs are reported in µg/g on a wet weight basis using percent moisture content.

As part of earlier research studies examining health effects and exposure in herring gulls in the Thunder Bay AOC, eggs were collected from Mutton Island for several years since 1992 and analyzed for contaminants (ECCC unpublished). Compared to current data, these early data allow for an assessment of temporal trends in contaminant exposure for birds foraging within the AOC. Double-crested cormorant eggs were also collected for contaminant analysis from Cone Island (48°13'17"N,

89°01'39"W) in 1989, a site that is just beyond the AOC boundary and east of Pie Island. All historic data are presented where available and compared to current burdens in herring gull embryos and cormorant eggs.

Stable Isotopes:

Stable isotope analyses of samples were conducted at the University of Ottawa's G.G. Hatch Stable Isotope Laboratory in Ottawa, Ontario. Following lipid-extraction, samples were weighed into tin capsules and loaded into an elemental analyser. The sample was flash combusted at ~1800°C (Dumas combustion) and the resultant gas products were carried by helium through columns of oxidizing/reducing chemicals optimised for CO_2 and N_2. The gases were separated by a purge and trap adsorption column and then sent to the Delta Advantage isotope ratio mass spectrometer coupled with Conflo III. Samples were normalized to internal standards and calibrated to international standards. Stable isotope ratios are expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following relationship:

$$\delta X = (R_{sample}-R_{standard})/R_{standard} \times 1000$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. In this study, $\delta^{15}N$ signatures were compared to infer relative (and not absolute) trophic position at colonies.

Feather Corticosterone:

Feathers from chicks were prepared by first removing the calamus, i.e., the proximal end of the quill to where the feathers start (~ 5 to 10 mm, depending on length of feather). The remaining portion was then minced with scissors until homogenous and 1.5 ml methanol was added. The sample was vortexed, sonicated for 30 minutes at room temperature, and incubated overnight at 50°C while shaking. After centrifuging at 13,000 rpm for 20 minutes, the supernatant was transferred to a fresh tube and the original feather sample was re-extracted with 1 ml methanol. This re-extraction was again vortexed for 5 minutes and the supernatant was removed and added to the original fraction. The entire methanol fraction was evaporated to dryness overnight in a fume hood and then the sample was reconstituted with 200 µl steroid diluent.

Corticosterone concentrations in the extracts of the feather sample were determined using a corticosterone EIA kit (Assay Designs – corticosterone enzyme immunoassay kit; product no. 900-097; 96 well kit). This is a competitive immunoassay for the quantitative determination of corticosterone in biological fluids and it uses a polyclonal antibody to corticosterone to bind in a competitive manner. In the presence of the corticosterone antibody, corticosterone in the sample competes with a known amount of corticosterone that has an alkaline phosphatase molecule covalently attached to it. The excess reagents are washed away and the substrate is added. Following a short incubation time, the enzyme reaction is stopped and the samples are read on a SpectraMax 190 UV-VIS microplate reader (Molecular Devices, Sunnyvale, California, USA) at 405 nm. The intensity of colour produced in the reaction is inversely proportional to the concentration of corticosterone in the sample. The measured optical density was used to calculate the concentration of corticosterone. The average net optical density (OD) bound for each standard and sample is calculated by subtracting the average NSB (non-

specific binding) OD from the average OD bound:

Average Net OD = Average Bound OD - Average NSB OD

Then the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo) was calculated using the following formula:

Percent Bound = Net OD x 100 Net Bo OD

The plot of percent bound versus concentration of corticosterone for the standards was graphed using Prism software (GraphPad, La Jolla, California, USA) and a line fitted through the points. The concentration of corticosterone in the sample was then determined. An in-house quality control sample was used in each plate. The sensitivity of the assay is 27 ρg/ml.

Thyroid Status:

Blood samples were centrifuged for 5 minutes at 14,000 rpm to separate plasma from red blood cells. Plasma was stored at -80°C and red blood cells were stored at 4°C. Concentrations of free thyroxine in the plasma of chicks sampled in the three study years were determined using a commercially available kit (AccuBind Elisa microwells product no. 1225-300 - Monobind Inc., Lake Forest, CA, 92630, USA). The method is based on a competitive enzyme immunoassay format in which a competition is set up between an immobilized antibody, the enzyme-antigen conjugate and the free thyroxine in the sample. When equilibration is reached, the unbound antigen fraction is removed and the enzyme activity in the bound fraction is measured which is inversely proportional to the concentration of free thyroxine in the sample. As per the method, standards, controls and sample plasma, as well as the enzyme reagent, were added to the microplate wells, which contained the immobilized antibody. After an incubation period, the unbound fraction was washed from the wells and the substrate added. The reaction was stopped and the plate was read at 450 nm on a SpectraMax 190 UV-VIS microplate reader (Molecular Devices, Sunnyvale, California, USA). Concentrations were determined from the standard curve, which was fitted using a variable slope (four parameter) method using Prism software (GraphPad, La Jolla, California, USA).

Controls used were Randox product no. HS2611 assayed human sera levels 2 & 3 (Randox Laboratories Ltd., Antrim, UK). The method gave the intra-assay precision as 10.98%, 4.26%, and 3.25 % coefficient of variation for low, medium and high controls, respectively. The inter-assay precision was 10.81%, 6.01%, and 7.90 % coefficient of variation for low, medium, and high controls, respectively. The detection limit of this assay was 0.32 ng/dl.

Immune Response - Phytohemagglutinin (PHA) Skin Test:

Feathers were plucked from the patagium (“wing web”) of each wing in order to measure the thickness to the nearest 0.05 mm using pressure-sensitive calipers (Dyer 304-196). Two measurements were taken of each wing web and the mean of the two measurements was determined for each wing. To assist in measuring the same location on day 2, a small dot was made with a permanent marker at the site of measurement. Following the thickness determination, 0.1 ml of a 1 mg/ml solution of PHA (Sigma) dissolved in sterile phosphate buffered saline (PBS; 0.01M phosphate buffer, 0.137M NaCl, pH 7.4) was

injected intra- or sub-dermally into one wing web and 0.1 ml of PBS solution was injected into the other wing web as a control using a 1 ml syringe fitted with a 26 gauge intradermal needle. Injections of PHA and PBS were randomly alternated between right and left wings of birds tested. Twenty-four hours (±6 hours) after injection, the thickness of each wing web was re-measured in duplicate at the site of injection (indicated by marker dot) and the mean of the two measurements again determined. For each individual chick, the PHA stimulation index was calculated as the increase in wing web thickness caused by PHA in one wing web minus the increase caused by PBS in the other wing web. This test was conducted using chicks at study sites in 2015 only.

Statistical Analysis:

Contaminants and other biological endpoints were statistically analyzed using either the Student's *t*-test for between colony comparisons or a one-way ANOVA for among-colony comparisons, which when significant, was followed by Tukey's HSD test. Data were log-transformed (log_{10}) to meet conditions of equal variance and normality for parametric analysis. If data failed these assumptions, comparisons were made using either a Mann-Whitney U non-parametric test or Kruskal-Wallis one-way analysis of variance by ranks; post-hoc tests were conducted using non-parametric multiple comparison tests for unequal sample sizes. Concentrations of chemical and biochemical endpoints that were below the limit of detection were given a value of one-half of the detection limit. Since numbers of PCB and PBDE congeners differed depending on the year and laboratory performing the chemical analysis, statistical analysis of contaminant burdens among colonies were conducted using congeners that were common to all chemical analyses. For sum PCBs and sum PBDEs, this represented 35 and 14 common congeners, respectively. Mercury concentrations in samples were statistically analyzed on a dry weight basis; however, concentrations are reported on a wet weight basis for comparisons to published values and thresholds. A Spearman rank correlation analysis was performed to examine the relationship between the two stable isotopes in gull embryos. Data for productivity and the three health parameters relating to stress, thyroid status and immune response were combined for chicks from the two AOC colonies to increase sample size for comparisons to the reference colony. All results were considered significant at $p<0.05$.

Concentrations of 2,3,7,8-TCDD toxic equivalents (TEQs) were calculated for dioxin-like PCBs, furans, and dioxins and are based upon toxic equivalency factors developed by van den Berg *et al.* (1998) for birds. Dioxin-like PCBs include four non-*ortho* PCB congeners (77, 81, 126, and 169) and eight mono-*ortho* PCB congeners (105, 114, 118, 123, 156, 157, 167, and 189). Of the eight mono-*ortho* PCB congeners, three were quantified in all analyses (105, 118, and 156), three were quantified in some analyses (114, 157 and 189) and two were not quantified in any analyses (123 and 167). Mean mono-*ortho* PCB concentrations, quantified in the chemical analysis for organochlorines, were calculated for individuals used to create the pool (where data were available) for reporting of TEQs associated with mono-*ortho* PCBs. Total TEQ concentration is based on the sum concentration of TEQs calculated for the 4 non-*ortho* PCBs, 3 or 6 mono-*ortho* PCBs (where data were available), and 17 dioxin and furan congeners.

RESULTS

A) Artificial Egg Incubation Study

Embryonic Viability and Deformities:

Embryonic viability was consistently high at 92% overall in herring gulls from the Thunder Bay AOC and ranged between 83% to 100% at the two AOC colonies, Mutton Island and Welcome Islands-a, in 2012 and 2014 (Table 1). Embryonic viability was also high overall at 95% in gulls from the Double Island reference colony and equal to 96% and 93% in 2012 and 2013, respectively. Of 51 fertile eggs examined in 2012, a total of three embryos died: one from Mutton Island (early development; could not stage because the contents were rotten), one from Welcome Islands-a (stage 44) and one from Double Island (stage 29). Of 39 fertile eggs examined in 2013/4, two embryos died from Mutton Island (one very early, i.e., stage 6-8, and stage 33) and one embryo died from the reference colony (stage 36-37). No embryonic deformities (0%) were evident in incubated eggs from either of the AOC colonies or the reference colony in all study years. Overall, artificially incubated herring gull eggs collected from AOC colonies had high rates of embryonic viability and no embryonic deformities, which was similar to the results observed at the reference colony in the two study years.

Contaminants:

Concentrations of organochlorine compounds were generally low in herring gull embryos from the two AOC colonies and the reference colony in 2012 and 2014 with sum PCBs found at the highest concentrations (Table 2a). Mean sum PCB concentrations ranged from 0.75 µg/g in embryos from Welcome Islands-a in 2012 to 1.54 µg/g in embryos from Double Island in 2014. Mean concentrations of the remaining organochlorines in embryos were below 0.4 µg/g at the study colonies. Concentrations of *p,p'*-DDE in embryos were similar to or lower than sum PBDEs and, based on comparisons of means, were at least eight times higher than concentrations of the remaining organochlorines. The highest concentrations of sum PCBs (2.83 µg/g) and *p,p'*-DDE (0.82 µg/g) in this study were found in an embryo from Double Island in 2014. The maximum sum PBDE concentration (4.84 µg/g) was also found in an embryo from Double Island with the next highest concentration (1.48 µg/g) in an embryo from Mutton Island in 2014. Significant differences in concentrations of two organochlorines were found in herring gull embryos among the three colonies in 2012 and for several more compounds, including PCBs, in 2014. Noteworthy is that for compounds where this was evident, mean concentrations in embryos from the Double Island reference colony were significantly higher than concentrations in either one or both of the AOC colonies. Mean percent lipid content was not significantly different among colonies within a study year and ranged from 7.2% in embryos from Mutton Island in 2014 to 8.7% in embryos from Double Island in 2014. Organochlorine and sum PBDE burdens in herring gull embryos from AOC colonies were overall comparable to or lower than burdens in embryos from the Double Island reference colony.

Concentrations of organochlorines and sum PBDEs in double-crested cormorant eggs were also consistently lower in pooled samples from the AOC colony on Welcome Islands-a compared to the Black Rock reference colony in the two study years (Table 2b). Sum PCBs in cormorant eggs were found at the highest concentrations (1-3 µg/g) followed by *p,p'*-DDE (1 µg/g), sum PBDEs (0.05 µg/g) and then the remaining organochlorines. Comparing burdens between the two colonial waterbird species at Welcome

Table 1. Embryonic viability and incidence of embryonic deformities in artificially incubated herring gull eggs collected from Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a) in 2012 and 2014 and the reference colony (Double Island) in 2012 and 2013*.

Colony	AOC/ Ref	Year	Total No. Eggs	No. Infertile Eggs	No. Fertile Eggs	No. Viable Eggs	No. Dead Eggs	Embryonic Viability (%)	No. Deformities	Deformities (%)
Mutton I.	AOC	2012	15	2	13	12	1	92%	0	0%
		2014	15	3	12	10	2	83%	0	0%
Welcome I.-a	AOC	2012	15	2	13	12	1	92%	0	0%
		2014	15	3	12	12	0	100%	0	0%
Overall	**AOC**		**60**	**10**	**50**	**46**	**4**	**92%**	**0**	**0%**
Double I.	Ref	2012	26	1	25	24	1	96%	0	0%
		2013	15	0	15	14	1	93%	0	0%
Overall	**Ref**		**41**	**1**	**40**	**38**	**2**	**95%**	**0**	**0%**

*Due to late ice-out conditions and the inability to access the Double Island reference colony during the early-laying period in 2014, the results of the artificial incubation study conducted in 2013 were used.

Table 2. Concentrations of organochlorines and sum PBDEs in embryos of herring gulls (a) and eggs of double-crested cormorants (b) from Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a) and corresponding reference colonies (Double Island or Black Rock) in 2012 and 2014 (µg/g, wet weight). Mean concentrations (SD) in herring gulls are based on analysis of ten individual embryos following incubation in the lab while concentrations in double-crested cormorant eggs are based on analysis of a single pool consisting of 10 or 13 eggs. Different uppercase letters indicate significant differences among gull colonies within a study year. Statistical comparisons of sum PCBs and sum PBDEs in 2012 are based on congeners that were common among colonies, i.e., the sum of 35 PCB congeners and 14 PBDE congeners, respectively.

a) Herring gull embryos:

Colony	AOC/ Ref	Year	*p,p'*-DDE	Sum Chlordane	HCB	Dieldrin	HE	Mirex	OCS	PCB 1:1[a]	Sum PCBs[b]	Sum PBDEs[c]
Mutton I.	AOC	2012	0.261 (0.076)	0.023 (0.011)	0.008 (0.006)	0.007 (0.005)	0.006 (0.002)	0.005 (0.006)**AB**	0.0007 (0.0010)**AB**	NA	0.746 (0.241)	0.258 (0.084)
Welcome I.-a	AOC	2012	0.227 (0.135)	0.021 (0.012)	0.005 (0.002)	0.008 (0.012)	0.011 (0.012)	0.003 (0.002)**B**	0.0003 (0.0004)**B**	NA	0.745 (0.419)	0.286 (0.152)
Double I.	Ref	2012	0.218 (0.048)	0.025 (0.005)	0.008 (0.005)	0.007 (0.005)	0.006 (0.001)	0.010 (0.013)**A**	0.002 (0.002)**A**	2.187 (1.177)	1.131 (0.704)	0.332 (0.185)
Mutton I.	AOC	2014	0.258 (0.195)	0.027 (0.015)**B**	0.009 (0.005)**B**	0.017 (0.019)**AB**	0.008 (0.004)**B**	0.017 (0.026)	0.001 (0.001)**B**	2.730 (1.161)	1.068 (0.457)**AB**	0.689 (0.394)
Welcome I.-a	AOC	2014	0.246 (0.129)	0.030 (0.015)**AB**	0.007 (0.002)**B**	0.007 (0.003)**B**	0.008 (0.003)**B**	0.006 (0.004)	0.0009 (0.0002)**B**	2.417 (0.990)	0.862 (0.317)**B**	0.379 (0.194)
Double I.	Ref	2014	0.374 (0.199)	0.046 (0.016)**A**	0.019 (0.015)**A**	0.022 (0.014)**A**	0.013 (0.004)**A**	0.033 (0.048)	0.004 (0.003)**A**	3.828 (1.800)	1.543 (0.768)**A**	0.787 (1.429)

b) Double-crested cormorant eggs:

Colony	AOC/ Ref	Year	*p,p'*-DDE	Sum Chlordane	HCB	Dieldrin	HE	Mirex	OCS	PCB 1:1[a]	Sum PCBs[b]	Sum PBDEs[c]
Welcome I.-a	AOC	2012	1.337	0.010	0.006	0.024	0.007	0.003	0.001	NA	0.921	0.036
Black Rock	Ref	2012	1.540	0.028	0.043	0.042	0.018	0.009	0.004	5.480	3.370	0.114
Welcome I.-a	AOC	2014	1.070	0.014	0.008	0.028	0.008	0.008	0.001	2.320	1.180	0.044
Black Rock	Ref	2014	1.470	0.016	0.009	0.032	0.008	0.006	0.003	3.570	1.470	0.054

[a] Based on 1:1 ratio of Aroclor 1254:1260; NA denotes that data are not available

[b] Based on sum concentrations of 35-62 PCB congeners

[c] Based on sum concentration of 15 or 25 PBDE congeners

Islands-a, concentrations of all organochlorines and sum PBDEs in cormorant eggs were within the ranges of concentrations in gull embryos in 2012 and 2014 with the exception of *p,p'*-DDE where concentrations in pooled cormorant eggs were at least two times higher than the maximum concentration in gull embryos.

Concentrations of four non-*ortho* PCBs, 2,3,7,8-TCDD, and total TEQs were lower in embryos from the Thunder Bay AOC colonies compared to the Double Island reference colony in 2011/2 and 2014 (Table 3a). Of the four non-*ortho* PCBs measured in herring gull embryos from study colonies in both years, concentrations of PCB-126 > 169 > 81 > 77. Overall, 2,3,7,8-TCDD concentrations were relatively lower in embryos from the AOC colonies in the two years (range=0.95-2.27 pg/g) compared to concentrations at the Double Island reference colony which were at least two times higher. Total TEQ concentrations ranged from 49.44 pg TEQ/g to 53.87 TEQ/g in embryos from AOC colonies and were below concentrations at the reference colony in the two years. Toxicity associated with non-*ortho* PCBs, dioxins and furans, and mono-*ortho* PCBs contributed (as means) 82%, 10%, and 8%, respectively, to the mean total TEQ concentration in embryos from AOC colonies in 2012 and 2014. Similar contributions of toxicity associated with non-*ortho* PCBs, dioxins and furans, and mono-*ortho* PCBs to mean total TEQ concentrations were found for embryos from the reference colony (as means, 79%, 14%, 8%, respectively). With respect to spatial patterns for TEQ burdens in herring gulls from colonies throughout the Great Lakes, embryos from the two Thunder Bay AOC colonies had the lowest mean TEQ concentration (51.89 pg TEQ/g; N=4 samples) in 2012 and 2014 compared to eggs collected annually from Great Lakes colonies from 2012-2014 (Figure 3). Results for artificially incubated herring gull embryos from the St. Marys River (Ontario) and eggs collected from Spanish Harbour in 2011 and 2012 are also provided for comparison purposes.

The total TEQ concentration in cormorant eggs from the AOC colony in 2014 was 78.64 pg TEQ/g which was nearly one half of that found in pooled eggs from the Black Rock reference colony (140.37 pg TEQ/g; Table 3b). Toxicity associated with non-*ortho* PCBs, dioxins and furans, and mono-*ortho* PCBs contributed 78%, 16%, and 6%, respectively, to the mean total TEQ concentration in eggs from the AOC colony. These relative contributions to total TEQ concentrations were also similar to those found at the reference colony. Comparing burdens between the two colonial waterbird species at Welcome Islands-a, concentrations of non-*ortho* PCBs, 2,3,7,8-TCDD and total TEQs were higher in cormorant eggs compared to gulls embryos in 2014.

Table 3. Concentrations of non-*ortho* PCBs, 2,3,7,8-TCDD, and 2,3,7,8-TCDD toxic equivalents (TEQ) in embryos of herring gulls (a) and eggs of double-crested cormorants (b) from Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a) and corresponding reference colonies (Double Island or Black Rock) in 2011/2 and 2014 (pg/g, wet weight). Each sample is a single pooled sample consisting of 5-15 embryos or 10 eggs. TEQs associated with 4 non-*ortho* PCBs, 17 dioxins and furans (PCDD/Fs), and 3 mono-*ortho* PCBs (#105, #118, and #156) and which together comprise total TEQs are also provided.

a) Herring gulls embryos:

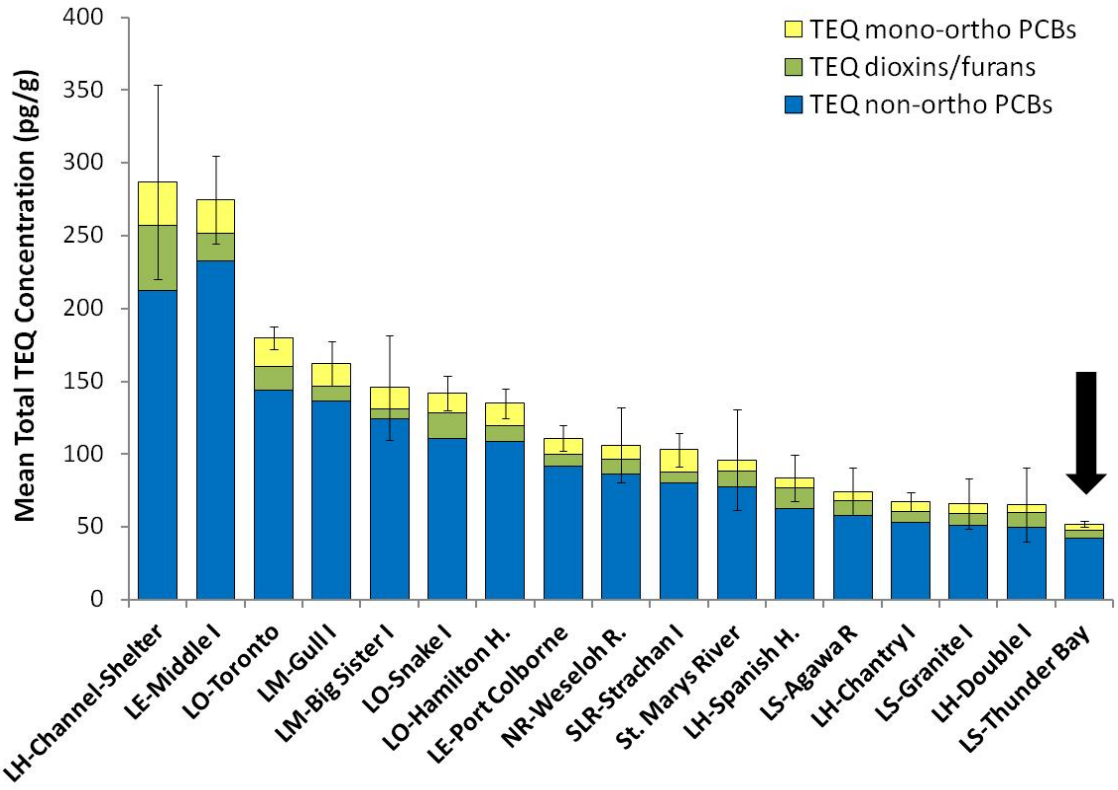
Colony	AOC/ Ref	Year	PCB-77	PCB-81	PCB-126	PCB-169	2,3,7,8 – TCDD	TEQ – non-*ortho* PCBs	TEQ – PCDD/Fs	TEQ – mono-*ortho* PCBs	Total TEQs
Mutton I.	AOC	2012	37.50	52.50	368.00	89.10	1.44	44.01	5.33	4.53	53.87
Welcome I.-a	AOC	2012	17.90	60.40	343.00	67.40	0.95	41.30	3.96	4.18	49.44
Double I.	Ref	2011	36.70	98.90	680.00	121.00	5.62	79.85	13.89	9.73*	103.46
Mutton I.	AOC	2014	12.00	49.60	373.00	71.40	2.27	42.93	6.06	4.50	53.49
Welcome I.-a	AOC	2014	9.06	33.20	381.00	105.00	0.97	41.98	4.98	3.79	50.75
Double I.	Ref	2014	64.40	73.10	570.00	120.00	4.93	67.65	11.93	5.41	84.99

* Includes 3 additional mono-*ortho* PCB congeners, #114, #157, and #189, since these data are available; combined these congeners contributed less than 10% to the total TEQ – mono-*ortho* PCB concentration.

b) Double-crested cormorant eggs:

Colony	AOC/ Ref	Year	PCB-77	PCB-81	PCB-126	PCB-169	2,3,7,8 – TCDD	TEQ – non-*ortho* PCBs	TEQ – PCDD/Fs	TEQ – mono-*ortho* PCBs	Total TEQs
Welcome I.-a	AOC	2014	85.60	134.00	438.00	102.00	3.07	61.58	12.24	4.81	78.64
Black Rock	Ref	2014	291.00	146.00	797.00	138.00	6.23	108.99	25.31	6.08	140.37

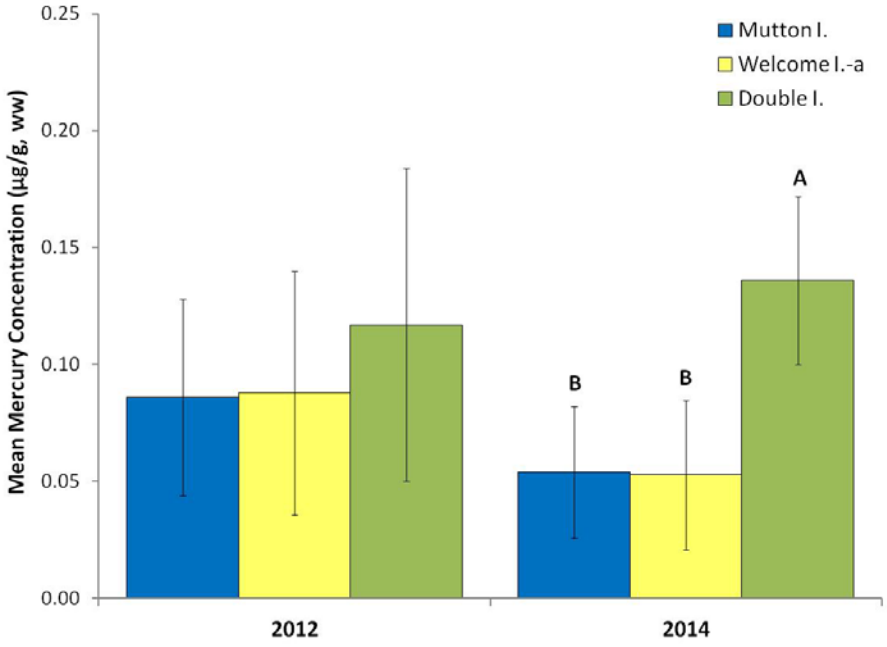
Figure 3. Mean total TEQ concentrations (SD) in embryos (N=4 samples total) from Thunder Bay AOC colonies in 2012 and 2014 and in herring gull eggs from Great Lakes colonies from 2012-2014 (pg/g, wet weight). Data for St. Marys River (Ontario) are based on artificially incubated herring gull embryos (N=6 samples) and for Spanish Harbour are based on eggs collected in 2011 and 2012 (N=2 pools). The contributions of TEQ concentrations associated with mono-*ortho* PCBs, dioxins and furans, and non-*ortho* PCBs to the total TEQ concentration are shown. Means are arranged in decreasing order from highest to lowest concentrations. Colony locations are associated with the following lakes/rivers: LH=Lake Huron, LO=Lake Ontario, LE=Lake Erie, SLR=St. Lawrence River, NR=Niagara River, LS=Lake Superior, and LM=Lake Michigan.



Mean mercury concentrations (SD) in herring gull embryos in 2012 were statistically similar among the three study colonies ranging from 0.09 (0.04) µg/g at Mutton Island to 0.12 (0.07) µg/g wet weight at Double Island (Figure 4a). In 2014, some spatial differences among colonies were evident with mean mercury concentrations (SD) ranging from 0.05 (0.03) µg/g at Welcome Islands-a to 0.14 (0.04) µg/g at Double Island ($F_{2,27}$=16.58, p=0.00002). Mercury concentrations in embryos from Double Island were significantly higher than concentrations in embryos from Mutton Island and Welcome Islands-a, which were not significantly different from each other. The maximum mercury concentration (0.28 µg/g) was found in an embryo from Double Island in 2012. Relative to mercury concentrations in herring gull eggs from other Great Lakes colonies from 2012 to 2014, mean mercury concentrations in gull embryos from the two AOC colonies were the lowest of 17 herring gull colonies monitored (Figure 5; ranked as means from highest to lowest; ECCC unpublished). Similar to the TEQ data presented as a broad comparison of Great Lakes trends, results for herring gull embryos incubated in the lab from the St. Marys River (Ontario) and for eggs collected from Spanish Harbour in 2011 and 2012 are also included here.

Figure 4. Concentrations of total mercury (µg/g, wet weight) in embryos of herring gulls (a) and eggs of double-crested cormorants (b) from Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a) and corresponding reference colonies (Double Island or Black Rock) in 2012 and 2014. Mean concentrations (SD) in herring gulls are based on 10 or 15 embryos per colony. Different uppercase letters show significant differences in mean concentrations among gull colonies within a study year and are based on statistical analysis of dry weight mercury concentrations. Concentrations in double-crested cormorant eggs are based on analysis of a single pool consisting of 10 or 13 eggs.

a) Herring gulls embryos:



b) Double-crested cormorant eggs:

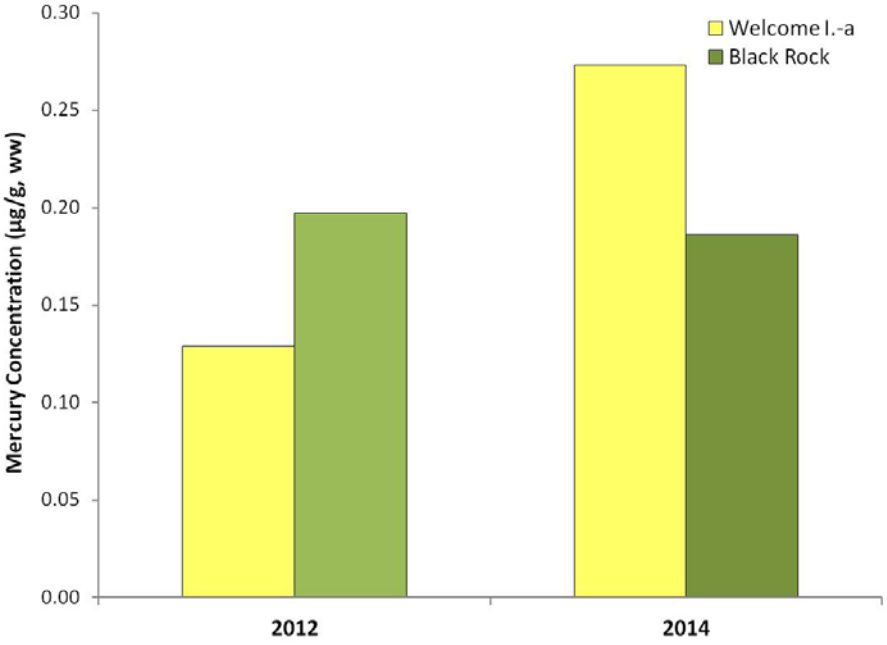
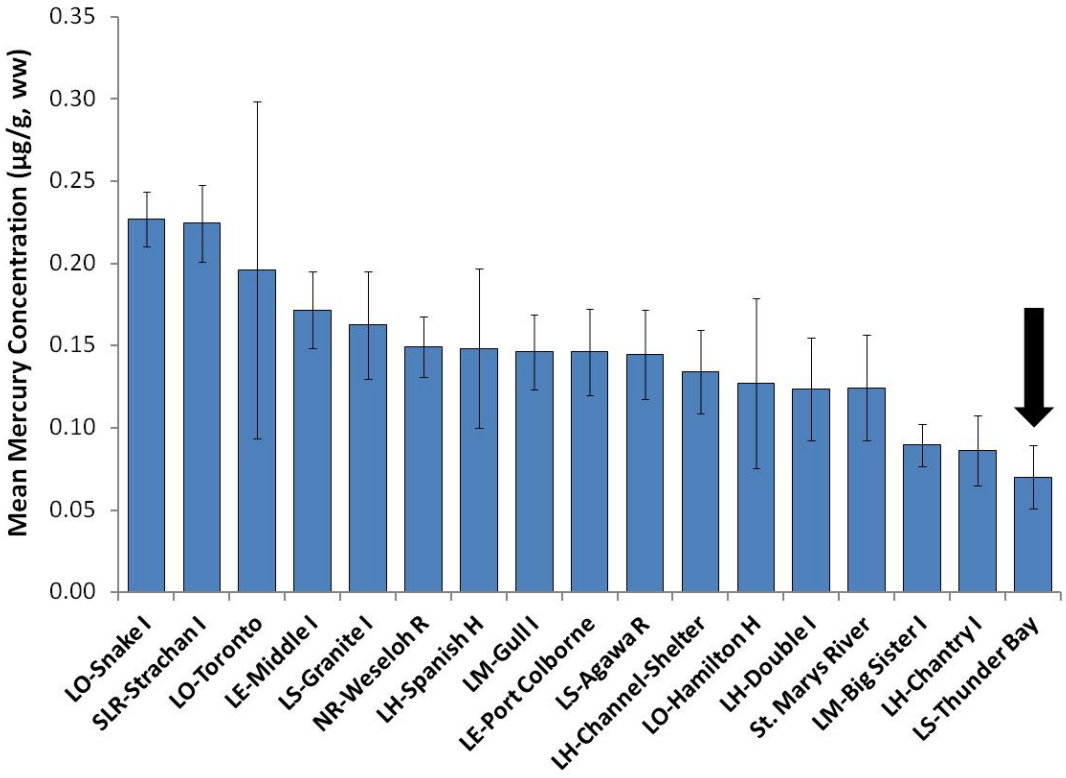


Figure 5. Mean mercury concentrations (SD) in embryos from Thunder Bay AOC colonies in 2012 and 2014 (based on N=4 mean concentrations) and in herring gull eggs from Great Lakes colonies from 2012-2014 (µg/g, wet weight). Data for St. Marys River (Ontario) are based on artificially incubated herring gull embryos (N=4 mean concentrations) and for Spanish Harbour are based on eggs collected in 2011 and 2012 (N=2 pools). Codes for lakes and rivers associated with colony locations are provided in caption for Figure 3.



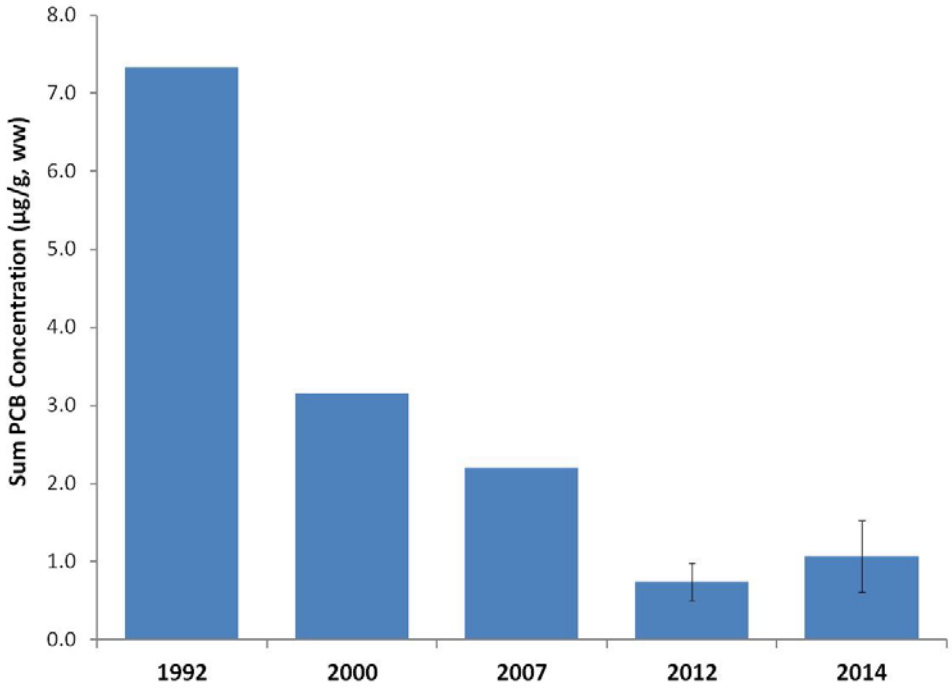
Mercury concentrations in double-crested cormorant eggs (as pooled samples) from Welcome Islands-a were 0.13 µg/g in 2012 and 0.27 µg/g in 2014 (Figure 4b). Concentrations of mercury in eggs from Black Rock were more similar between years and equal to 0.20 µg/g and 0.19 µg/g in 2012 and 2014, respectively. No consistent trends were apparent between AOC and reference colonies in the two study years. Comparing burdens between the two colonial waterbird species at Welcome Islands-a, the concentration of mercury in cormorant eggs was within the range of concentrations in gull embryos in 2012 but not in 2014 when the concentration in eggs was over two times higher than the maximum concentration in gulls embryos.

Temporal Trends of Contaminants in Herring Gulls and Cormorants:

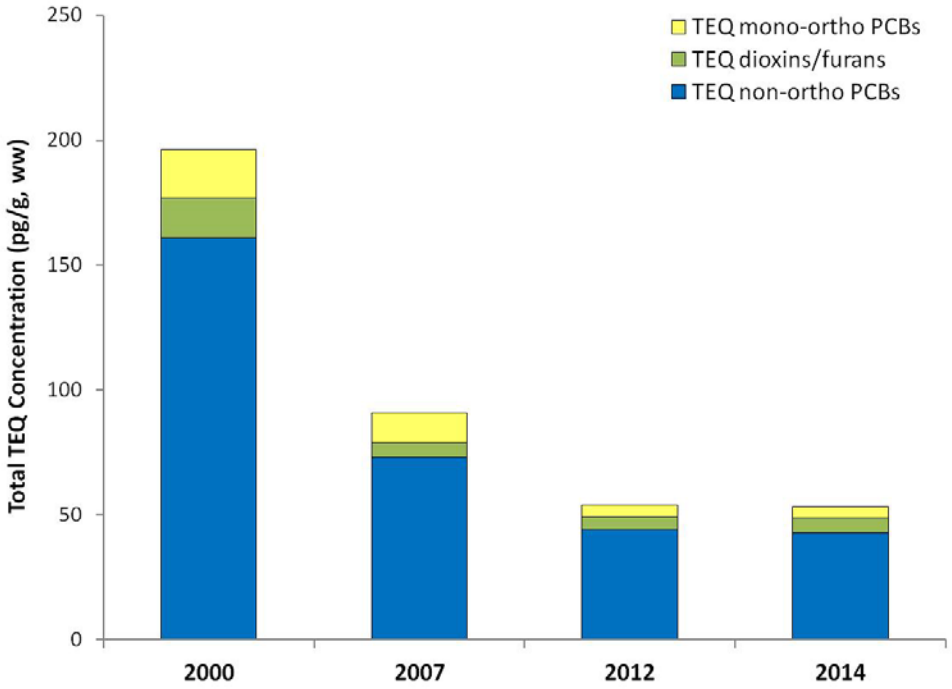
Concentrations of sum PCBs in herring gulls from Mutton Island declined dramatically in the five study years when eggs were collected from the Thunder Bay AOC. Overall, sum PCBs decreased by 85.4% based on an initial concentration of 7.33 µg/g in eggs in 1992 (analyzed as a single pool) and a mean concentration of 1.07 µg/g in embryos in 2014 (Figure 6a). With the exception of OCS, large decreases of at least 56% were also found for concentrations of other organochlorines and 2,3,7,8-TCDD between these two years (Supplementary Information, Table S1). Concentrations of total TEQs also declined

Figure 6. Temporal trends for concentrations of sum PCBs, total TEQs and mercury in herring gull eggs and embryos from Mutton Island in the Thunder Bay AOC in 1992, 1993, 1996, 2000, 2007, 2012 and 2014 (where data are available). Depending on the compound analyzed, concentrations are based on analysis of a single pooled sample of eggs or embryos or as means (SD) of two pools of eggs (1993 and 1996) or 10 individual embryos (2012 and 2014).

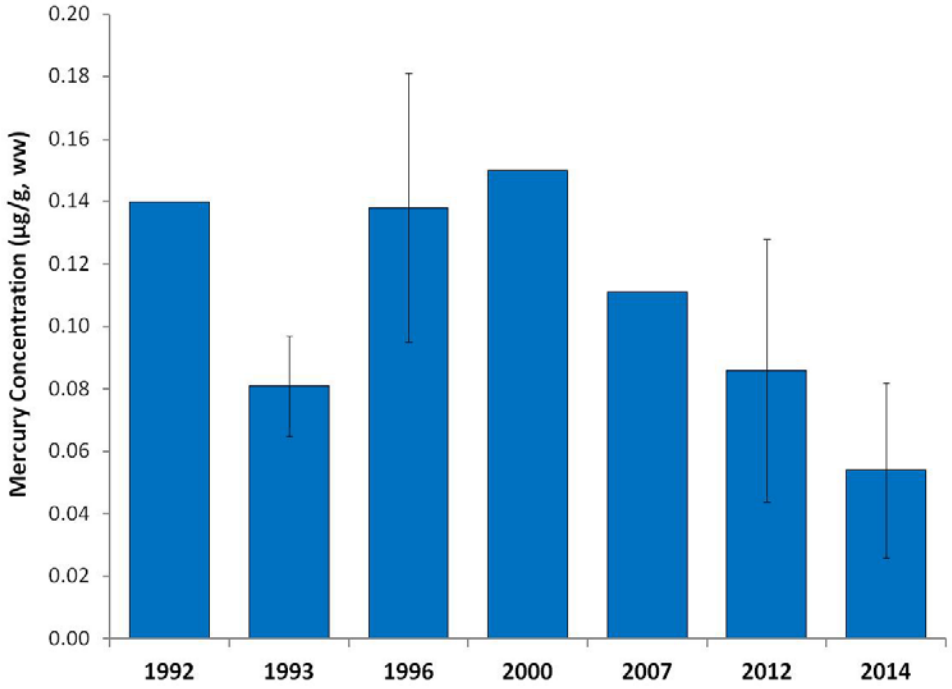
a) Sum PCBs



b) Total TEQs:



c) Mercury



steadily from 196.22 pg TEQ/g in eggs in 2000 to 53.49 pg TEQ/g in embryos in 2014 representing a decrease of 72.7% between these two years (Figure 6b). A temporal decline in mercury concentrations was not evident in gulls when eggs were collected from Mutton Island over seven years from 1992 to 2014 and concentrations varied between years. Mercury concentrations ranged from 0.05 µg/g (wet weight) in embryos in 2014 to 0.15 µg/g in eggs collected in 2000 and analyzed as a pooled sample (Figure 6c; Supplementary Information, Table S2).

Large decreases in contaminant burdens were also evident in cormorant eggs collected from Cone Island (just beyond the boundary of the AOC) in 1989 relative to current concentrations reported in eggs collected from Welcome Islands-a. Sum PCB burdens in cormorants decreased by 70.8% from an initial concentration of 4.04 µg/g in eggs in 1989 (analyzed as a single pool) to 1.18 µg/g in eggs from Welcome Islands-a in 2014 (Supplementary Information, Table S1). Similar large decreases were found for other organochlorines in cormorant eggs between these two years (range=39%-91%). Concentrations of 2,3,7,8-TCDD also decreased by 74.4% from an initial concentration of 12 pg/g in cormorant eggs in 1989 to 3.07 pg/g in eggs in 2014.

Stable Isotopes:

Significant spatial differences for mean $\delta^{15}N$ values in herring gull embryos were found among study colonies in 2014 only (H=12.32, p=0.002; Table 4a). As an indicator of trophic position, mean $\delta^{15}N$ values were significantly higher in gulls from the Double Island reference colony compared to both AOC colonies. A similar spatial trend was evident in gull embryos in 2012, however, this was not statistically significant. Mean $\delta^{13}C$ values in gull embryos from Welcome Islands-a were significantly more depleted (i.e., more negative) than mean values in embryos from the other AOC colony in 2012, while values at

the Double Island reference colony were not significantly different from either of the two AOC colonies (H=12.17, p=0.002). No significant differences for mean $\delta^{13}C$ values were found in 2014 among colonies. No significant correlations were found between $\delta^{15}N$ and $\delta^{13}C$ values when herring gull embryos from all colonies and years were grouped together or when examined separately by colony and year.

Table 4. Values for $\delta^{15}N$ and $\delta^{13}C$ in embryos of herring gulls (a) and eggs of double-crested cormorants (b) collected from Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a) and corresponding reference colonies (Double Island or Black Rock) in 2012 and 2014. Mean (SD) values in herring gulls are based on 10-15 embryos per colony. Different uppercase letters show significant differences in mean values among gull colonies within a study year. Values in double-crested cormorant eggs are based on analysis of a single pool consisting of 10 or 13 eggs.

a) Herring gull embryos:

Colony	AOC/Ref	$\delta^{15}N$		$\delta^{13}C$	
		2012	2014	2012	2014
Mutton I.	AOC	9.16 (0.35)	8.76 (0.38) **B**	-21.14 (0.34) **A**	-21.14 (0.55)
Welcome I.-a	AOC	9.28 (0.48)	8.74 (0.33) **B**	-21.98 (0.34) **B**	-21.32 (0.54)
Double I.	Ref	9.47 (1.03)	9.95 (1.05) **A**	-21.43 (0.79) **AB**	-21.60 (0.69)

b) Double-crested cormorant eggs:

Colony	AOC/Ref	$\delta^{15}N$		$\delta^{13}C$	
		2012	2014	2012	2014
Welcome I.-a	AOC	11.56	13.27	-24.59	-22.31
Black Rock	Ref	13.20	13.26	-21.20	-22.34

Stable isotope values in cormorant eggs varied more between years at the AOC colony compared to the Black Rock reference colony in the two study years (Table 4b). Comparing isotopic signatures between the two colonial waterbird species at Welcome Islands-a, cormorants occupied a higher trophic position with $\delta^{15}N$ values that were higher than all gull embryos. They also had more depleted carbon values in eggs in 2012 and 2014 compared to gulls in the two study years.

B) Field Study

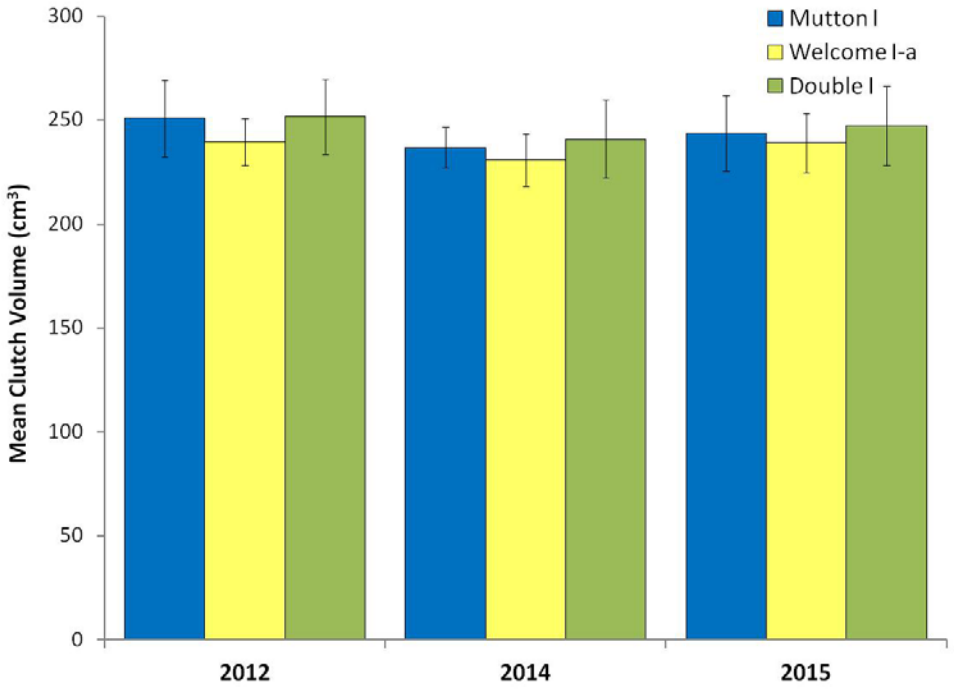
Egg Size Parameters:

To assess potential food stress in birds, total clutch volume and intraclutch variation in egg size were examined in 3-egg clutches in 2012, 2014, and 2015. Mean total clutch volume (SD) in herring gull eggs from Thunder Bay AOC colonies ranged from 231.0 (12.7) cm^3 at Welcome Islands-a in 2014 to 251.0 (18.3) cm^3 at Mutton Island in 2012 (Figure 7a). Total clutch volume in gull eggs at the Double Island colony ranged from 241.0 (18.7) cm^3 in 2014 to 251.7 (18.1) cm^3 in 2012. Mean total clutch volumes were not significantly different among clutches from AOC colonies and the Double Island reference colony in the three study years.

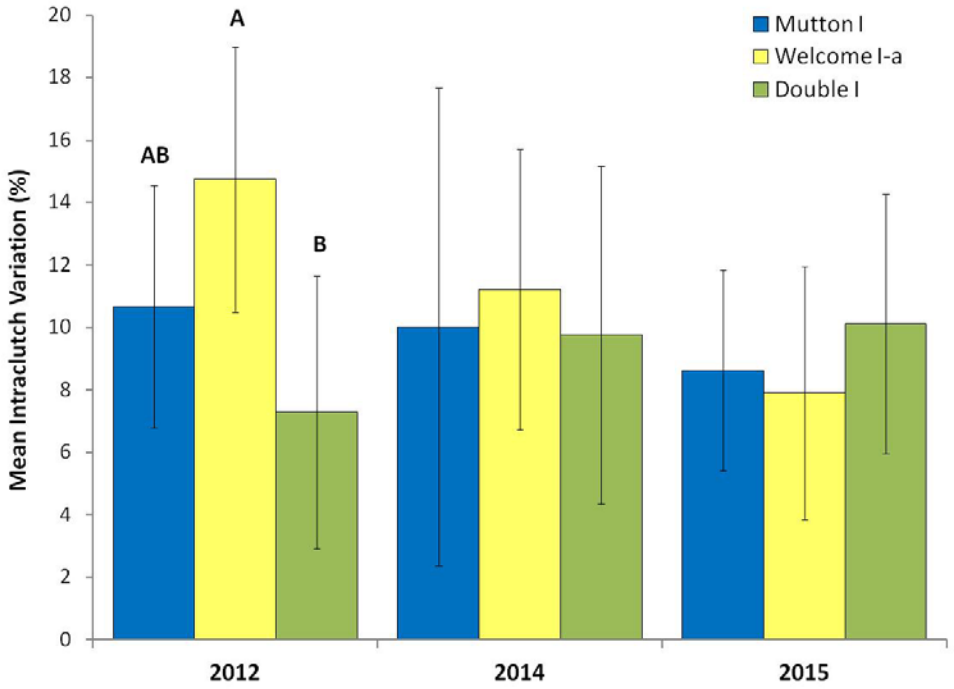
Mean intraclutch variation (SD) in egg size at AOC colonies ranged from 7.9 (4.1)% at Welcome Islands-a in 2015 to 14.7 (4.3)% at Welcome Islands-a in 2012. In 2012, mean intraclutch variation was

Figure 7. Total clutch volume (a) and intraclutch variation (b) in 3-egg clutches of herring gulls from Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a) and the reference colony (Double Island) in 2012, 2014, and 2015. Numbers of 3-egg clutches ranged from 8-42 at each colony. Different uppercase letters show significant differences in mean estimates within a study year.

a) Mean total clutch volume (SD):



b) Mean intraclutch variation (SD) in egg size:

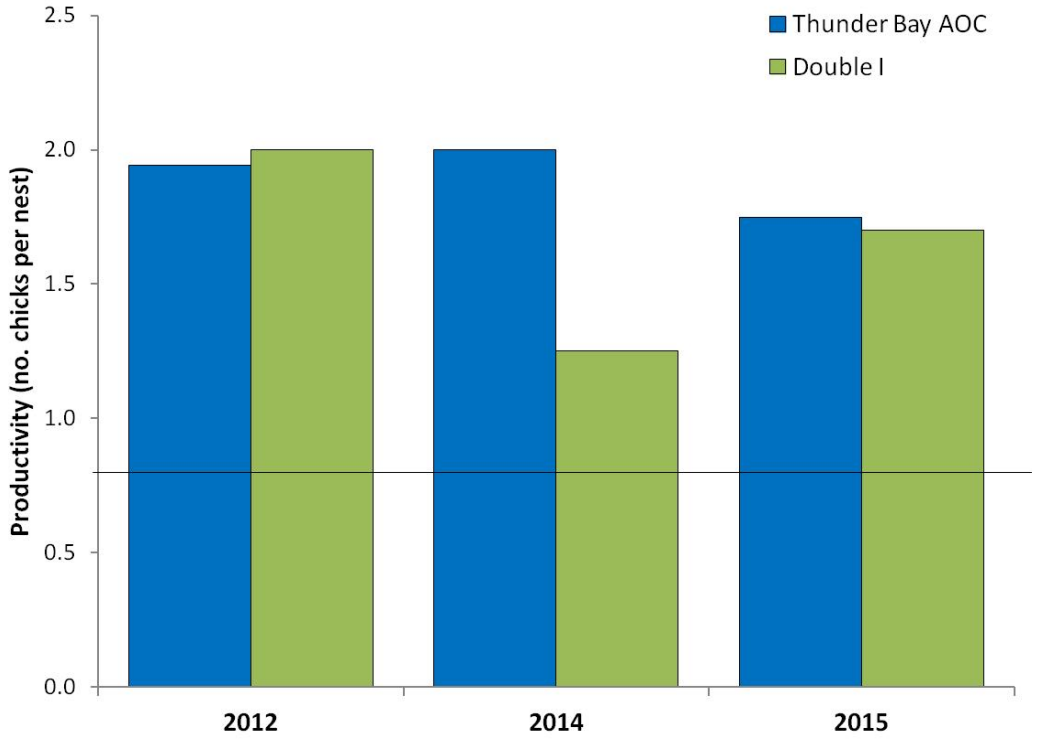


significantly different among colonies ($F_{2,51}$=13.66, p=0.00002; Figure 7b) and was significantly higher between clutches from Welcome Islands-a compared to Double Island with clutches from Mutton Island not significantly different from either of the two colonies. No significant differences were found for this parameter among study sites in either 2014 or 2015.

Productivity & Prevalence of Deformities in Wild Populations:

In 2012, herring gull productivity, defined as the number of ≥ 21-day-old chicks produced per nest, was equal to 1.9 chicks per nest at the two Thunder Bay AOC colonies (N=17 enclosed nests, combined) and 2.0 chicks per nest at Double Island (N=7 nests; Figure 8). Productivity was consistently high at AOC colonies in 2014 and 2015 and equal to 2.0 chicks per nest (N=5 enclosed nests) and 1.8 chicks per nest (N=8 enclosed nests), respectively. Productivity at the reference colony was slightly lower at 1.3 chicks per nest in 2014 and 1.7 chicks per nest in 2015 (N=8 and 10 enclosed nests, respectively). Overall, productivity estimates at AOC colonies in the three study years were well within the range of productivity levels required to maintain a stable population (0.8-1.4 chicks per nest; Kadlec and Drury 1968). In addition, many herring gull chicks were observed at the colony across the channel at Welcome Islands-b in 2015. A formal survey was not conducted on this island due to concerns that disturbance might impact a colony of American white pelicans (*Pelecanus erythrorhynchos*) that were also observed nesting there.

Figure 8. Herring gull productivity, as the number of ≥ 21-day-old chicks produced per nest, at Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a, combined) and the reference colony (Double Island) in 2012, 2014 and 2015. The solid line indicates the minimum productivity level of 0.8 chicks per nest associated with maintaining a stable herring gull population (range in levels=0.8-1.4 chicks per nest; Kadlec and Drury 1968).



In 2012, 2014 and 2015, no morphological deformities were found in herring gull chicks from enclosed nests at the two AOC colonies (combined) or the reference colony (N=10-33 chicks; Table 5).

Table 5. Prevalence of morphological deformities (%) in herring gull chicks examined in enclosed nests from Thunder Bay AOC colonies (Mutton Island & Welcome Islands-a, combined) and the reference colony (Double Island) in 2012, 2014 and 2015.

Colony	Year	No. Chicks Examined	% Deformities
Thunder Bay AOC	2012	33	0%
	2014	10	0%
	2015	14	0%
Double I.	2012	14	0%
	2014	10	0%
	2015	17	0%

Corticosterone in Feathers of Herring Gull Chicks:

Mean corticosterone concentrations (SD) in feathers of herring gull chicks from Thunder Bay AOC colonies (combined) ranged from 0.86 (0.65) ρg/mm to 1.69 (0.11) ρg/mm in 2012, 2014 and 2015 (Figure 9). Mean corticosterone concentrations (SD) in feathers from the Double Island reference colony ranged from 2.01 (2.44) ρg/mm to 2.77 (4.11) ρg/mm in the three study years. Significant spatial differences were found between the Thunder Bay AOC colonies and the reference colony in two of three study years in which mean corticosterone concentrations were significantly higher at the reference colony compared to the AOC colonies ($t>2.10$, $p<0.05$). Relative to AOC chicks, these results suggest that chicks from the reference colony experienced higher levels of stress during the period of feather growth.

Thyroxine in Plasma of Herring Gull Chicks:

Mean thyroxine concentrations (SD) in plasma of herring gull chicks from Thunder Bay AOC colonies (combined) ranged from 0.24 (0.07) ng/dl to 0.69 (0.29) ng/dl in 2012, 2014 and 2015 (Figure 10). Mean thyroxine concentrations (SD) in plasma of chicks from Double Island were comparatively lower and ranged from 0.19 (0.07) ng/dl to 0.59 (0.39) ng/dl in the three study years. Mean thyroxine concentrations were significantly higher in plasma of chicks from AOC colonies compared to the reference colony in 2012 (Mann Whitney test $U=98.50$, $p=0.014$). No significant differences in thyroxine concentrations were found between sites in 2014 and 2015.

Figure 9. Mean corticosterone concentrations (SD), expressed as picograms of corticosterone per millimetre of feather, in herring gull chicks from Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a, combined) and the reference colony (Double Island) in 2012, 2014 and 2015. Numbers of chicks sampled ranged from 4-29 per site. Different uppercase letters show significant differences in mean concentrations within a study year.

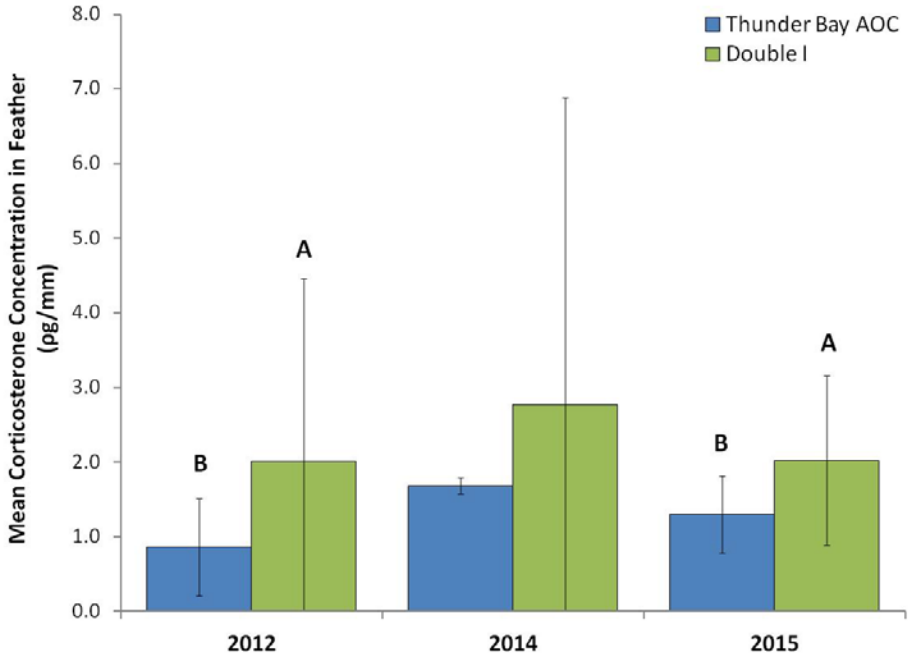
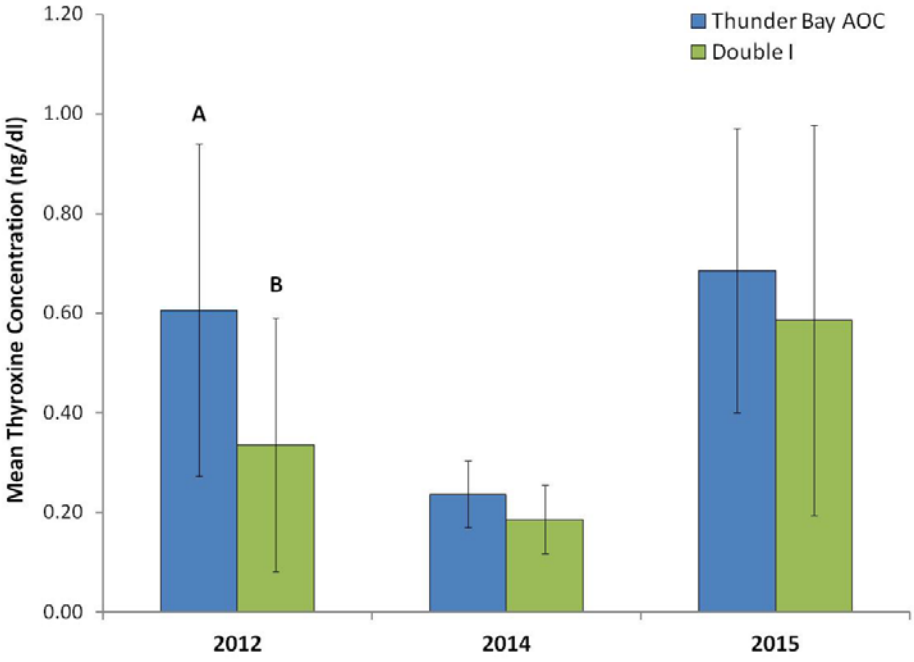


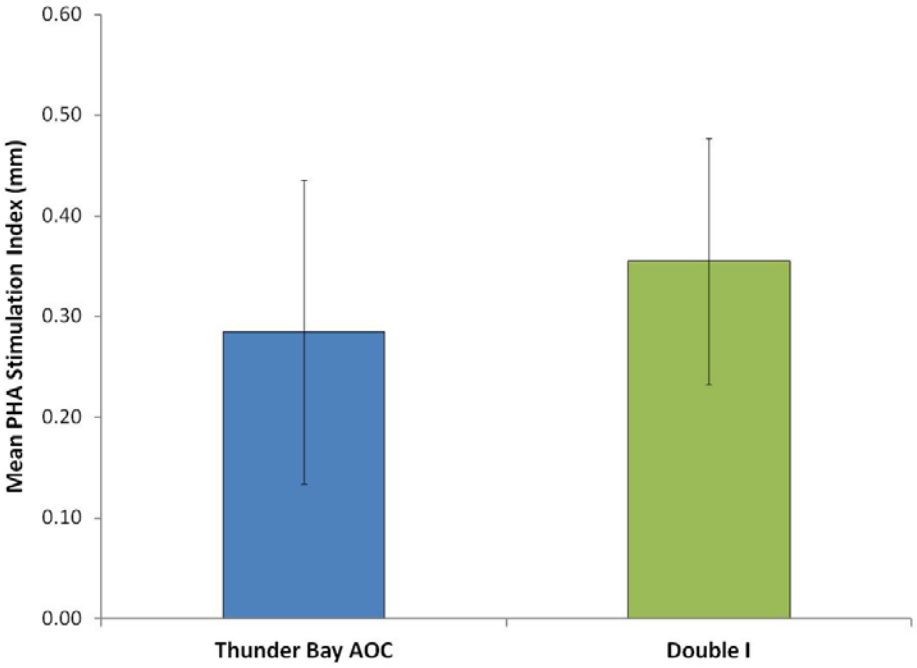
Figure 10. Mean thyroxine concentrations (SD) in plasma of herring gull chicks from Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a, combined) and the reference colony (Double Island) in 2012, 2014 and 2015 (ng/dl). Numbers of chicks sampled ranged from 5-29 per site. Different uppercase letters show significant differences in mean concentrations within a study year.



Immune Response in Herring Gull Chicks:

Estimates of the PHA stimulation index as means (SD) were 0.28 (0.15) mm and 0.36 (0.12) mm for chicks from the Thunder Bay AOC colonies (combined) and Double Island reference colony, respectively, in 2015 (Figure 11). There was no significant difference in PHA response in herring gull chicks between the two sites.

Figure 11. Mean PHA stimulation index (SD) in herring gull chicks from Thunder Bay AOC colonies (Mutton Island, Welcome Islands-a, combined) and the reference colony (Double Island) in 2015. Fourteen chicks were tested at each site.



DISCUSSION

Using a multi-tiered lab and field study approach, assessments of reproduction and deformities in herring gulls nesting in the Thunder Bay AOC were conducted in three study years. Artificial incubation of freshly-laid eggs under controlled conditions in the laboratory were valuable for assessing the importance of intrinsic factors such as contaminants that may induce early embryonic mortality or result in developmental abnormalities at this critical life stage. In this study, embryonic viability in artificially incubated eggs from AOC colonies was consistently high in herring gulls and on average equal to 92% in 2012 and 2014. No embryonic deformities (0%) were evident in incubated eggs from AOC colonies in these two years. Egg viability in herring gulls from Mutton Island in 2007, based on field assessments, was also high and equal to 94.1% on average in three egg clutches (Supplementary Information, Table S3; ECCC unpublished).

Reproduction based on productivity values for herring gulls at two nesting colonies in the Thunder Bay AOC was considered good with productivity ranging from 1.8 to 2.0 chicks per nest in the three study years. These values were well above productivity levels required to maintain a stable herring gull

population (range=0.8-1.4 chicks per nest; Kadlec and Drury 1968). These results are also consistent with productivity estimates for herring gulls nesting at Welcome Islands-a and b determined in earlier research studies conducted in 2000 and 2008 (ECCC unpublished). Herring gull productivity at Welcome Islands-b in 2000 was estimated to be 1.1 chicks per nest based on total counts of 237 chicks at 216 nests. This value likely represents an underestimation since chicks can easily hide on the island or swim off the island, thus decreasing the census numbers. While no herring gulls nested on Welcome Islands-a in 2000, they nested on this island in 2008 and productivity was estimated to be 1.8 chicks per nest (21 chicks from 12 nests in a single large enclosure). Double-crested cormorant productivity was also estimated on Welcome Islands-a and b in 2000 (ECCC unpublished). Productivity was equal to 1.4 chicks per nest at the two colonies with 187 chicks counted at 131 nests at Welcome Islands-a and 584 chicks counted at 421 nests at Welcome Islands-b.

In this study, no morphological deformities were found in juvenile herring gull chicks from both AOC and reference colonies in 2012, 2014 and 2015. Similarly, no morphological deformities were found in gull and cormorant chicks examined as part of earlier research studies at Welcome Island-a and b in 2000 and 2008 when productivity was also assessed (as described above; ECCC unpublished). Two large-scale surveys of bill deformities in cormorant chicks were conducted on the Great Lakes by the Canadian Wildlife Service in 1979-1987 and 1988-1996 (Fox *et al.* 1991; Ryckman *et al.* 1998). Cormorant colonies assessed in both studies were largely located in the region encompassing the three large bays, including Thunder Bay, on the northwest shore of Lake Superior. Two chicks with bill deformities were found in total in these two surveys. Based on these results, the prevalence of bill defects in cormorant chicks from Lake Superior was 5.5 per 10,000 chicks at five colonies in 1979-1987 and 1.2 per 10,000 chicks at 18 colonies in 1988-1996. While these rates were not significantly different between the two time periods, the prevalence of chicks with bill deformities at Lake Superior colonies was relatively lower in the latter time period and was also the lowest of all of the Great Lakes where surveys were conducted (Ryckman *et al.* 1998).

Based on the low overall contaminant burdens reported in herring gull embryos and double-crested cormorant eggs in 2012 and 2014, concentrations of PCBs, other organochlorines and PBDEs were not sufficiently elevated to adversely impact the reproductive success of these species foraging in the Thunder Bay AOC. In a broad literature review of PCB effects in birds, Hoffman *et al.* (1996) concluded that sum PCB concentrations in the range of 8 to 25 µg/g in eggs were associated with decreased hatching success for terns and cormorants. Sum PCB concentrations in embryos and eggs of both species were well below the 8 µg/g threshold. Similarly, concentrations of *p*,*p*'-DDE in embryos and eggs were well below threshold levels associated with significant effects on reproductive success as reported in black-crowned night-herons (*Nycticorax nycticorax;* 8 µg/g; Henny *et al.* 1984) and cormorants (10 µg/g; Pearce *et al.* 1979). Sum PBDE concentrations were generally low in gulls from AOC colonies and below the lowest-observed effect level on pipping and hatching success in American kestrels (*Falco sparverius*) equal to 1.8 µg/g in eggs (McKernan *et al.* 2009). This threshold was exceeded in one gull embryo from the Double Island reference colony in 2014. Overall, concentrations of compounds in both species were largely comparable between AOC colonies and the reference colonies and for some compounds, e.g., PCBs and sum chlordane, were on occasion significantly lower in gulls at an AOC

colony than the respective reference colony in a study year.

Concentrations of 2,3,7,8-TCDD, dioxin-like PCBs and total TEQs in herring gull embryos and cormorant eggs from Thunder Bay AOC colonies were not notably elevated relative to birds from the respective reference colonies in 2011/2 and 2014. Total TEQs in herring gulls from the Thunder Bay AOC were the lowest of mean TEQ concentrations in herring gull eggs from other Great Lakes colonies, including those elsewhere on Lake Superior, from 2012-2014 (Figure 3). Mean concentrations of total TEQs and sum PCBs in gull embryos from Thunder Bay AOC colonies were also lower than median concentrations in herring gull eggs from three Lake Superior colonies in Michigan, one of which included a colony near Isle Royale, from 2008-2012 (Fuentes *et al.* 2014). Large declines in concentrations of sum PCBs and other organochlorines, 2,3,7,8-TCDD and total TEQs in herring gull tissues since the early 1990s indicate that exposure to these compounds has decreased in herring gulls foraging in the AOC (Figure 6; Supplementary Information, Table S1). These trends are consistent with larger scale declines reported for organochlorines and 2,3,7,8-TCDD in herring gull eggs collected from two Lake Superior colonies, Granite Island and Agawa Rocks, that have been monitored annually since the early 1970s (de Solla *et al.* 2016).

With respect to concentrations associated with toxicity, 2,3,7,8-TCDD (<4 pg/g) and total TEQs (<80 pg TEQ/g) in herring gull embryos and cormorant eggs from AOC colonies were below concentrations associated with effects on reproduction in avian species. Median concentrations of 2,3,7,8-TCDD (37 pg/g) and total TEQs (2175 pg TEQ/g) in eggs of Forster's tern (*Sterna forsteri*) from Lake Michigan were associated with a significant reduction in hatching success (TEQs based on concentrations of 2,3,7,8-TCDD and dioxin-like PCBs only; Kubiak *et al.* 1989). However, no effect on hatching success was observed at relatively lower median concentrations of 2,3,7,8-TCDD (8 pg/g) and total TEQs (201 pg TEQ/g) in eggs from the reference colony in 1983 (Kubiak *et al.* 1989). Total TEQ concentrations in gull embryos and cormorant eggs were also well below 1200-1300 pg TEQ/g at which high rates (6-10%) of embryonic deformities were found in cormorants at two Lake Michigan colonies in 1988 (Yamashita *et al.* 1993). Concentrations of 2,3,7,8-TCDD were three orders of magnitude below the lethal dose associated with 50% embryonic mortality in cormorants (4000 pg/g based on egg injection; Powell *et al.* 1998) and were well below those associated with decreased embryonic growth and edema in herons (150-250 pg/g; Hoffman *et al.* 1996). As effectively demonstrated in the artificial incubation component of this study, these compounds were not sufficiently elevated to impact embryonic viability in gull eggs from study colonies in the two years.

Exposure to high concentrations of mercury can have significant impacts on reproductive success in birds and result in teratogenic effects in avian embryos, as demonstrated in egg injection studies with methylmercury in the laboratory (Fimreite 1974; Hill *et al.* 2008; Heinz *et al.* 2011). Overall, mercury concentrations in all embryos were below the predicted threshold of 0.6 µg/g (wet weight) in eggs set to be protective against adverse reproductive effects for 95% of non-marine avian species (Shore *et al.* 2011). The highest mercury concentrations were approximately one-half of the threshold concentration and were found in a herring gull embryo from Double Island in 2012 and cormorant eggs (pooled sample) from Welcome Islands-a in 2014. Mercury burdens in gull embryos were not elevated at AOC compared to the references colonies and based on mean concentrations were the lowest of all Great

Lakes colonies where gull eggs were collected from 2012-2014 (Figure 5). Mean mercury concentrations in embryos from AOC colonies were also lower, i.e., one quarter, of median mercury concentrations in herring gull eggs from three Lake Superior colonies in Michigan from 2008-2012 (Fuentes *et al.* 2014). Low concentrations of mercury in gull embryos from AOC colonies are consistent with the normal embryonic development of artificially incubated eggs in the lab. While large and significant declines in mercury were found in herring gull eggs from the two Lake Superior colonies, Agawa Rocks and Granite Island, from 1974-2009, no significant changes in mercury concentrations were found at these colonies from 1994-2009 (Weseloh *et al.* 2011). This is similar to the temporal pattern found for mercury concentrations in eggs from the AOC during a comparable time span (Figure 6; Supplementary Information, Table S2). Overall, current mercury burdens were low and it is unlikely that mercury concentrations would impact reproduction of breeding colonial waterbirds in the Thunder Bay AOC.

Long-term population trends of nesting herring gulls and cormorants at AOC study colonies provide additional evidence that populations are not adversely impacted by conditions that are specific to the AOC. Using nest count surveys as an indicator of population trends of breeding herring gulls, total numbers of gull nests at Mutton Island and Welcome Islands-a and b increased from 450 nests in 1978 to 519 nests in 1999 to 868 nests in 2007 and then decreased to 757 nests in 2012 (Supplementary Information, Table S4). While gull nest numbers have not changed remarkably at each of the AOC colonies over 11 survey years (where survey data are available), total numbers of nests at the three AOC colonies were overall higher in 2007 and 2012 relative to nest numbers in 1978, 1989 and 1999. In contrast, numbers of cormorant nests at Welcome Islands-a and b increased dramatically from a single nest in 1978 to 1,574 nests in 2007. Lake-wide population trends based on decadal surveys conducted on the Canadian and American sides of Lake Superior indicate that nest numbers of herring gulls have fluctuated while cormorant nest numbers have increased steadily since the late 1970s (Supplementary Information, Table S4). Numbers of herring gull nests on Lake Superior were 13,269 in the first decadal survey and peaked to nearly 25,000 nests in the second survey. Nest numbers subsequently decreased to 18,987 in the third survey (1996-2001), and decreased again by nearly 20% to 15,255 nests in the fourth survey (2007-2010). Cormorant nests have increased steadily over the four surveys from 35 nests in 1978 to nearly 4,800 nests in 2007-2010 on Lake Superior. Over the four binational Great Lakes colonial waterbird decadal surveys, eight colonial waterbird species, including great blue heron (*Ardea herodias*) and common tern (*Sterna hirundo*), have been reported nesting on Lake Superior. Only two species, herring gulls and cormorants, have been found nesting within the Thunder Bay AOC since 1978 and both have been consistently recorded in years when surveys were conducted. A new colonizer to this area, the American white pelican, was observed breeding on Welcome Islands-b in 2015.

Stable isotopes of nitrogen and carbon are used to provide information on trophic position and carbon source in the food web, respectively (Hobson 1999). Significantly higher $\delta^{15}N$ values were found in gull embryos at the Double Island reference colony compared to AOC colonies in 2014 and a similar, albeit non-significant, trend was found in 2012. This suggests that gulls occupied a relatively higher trophic level at the reference colony compared to gulls from AOC study colonies. Specifically, gulls at this colony may have fed more on fish (or larger fish) compared to gulls from AOC colonies, which fed at a relatively lower trophic level and on a diet that may have included terrestrial food sources such as small mammals, refuse and plant material (Fox *et al.* 1990). Differences in trophic levels between colonies

may have contributed to higher concentrations of several organochlorines reported in gull embryos from the reference colony compared to AOC colonies. While a significant difference was found for $\delta^{13}C$ values in gull embryos between the two AOC colonies in 2012, no significant differences were found between either of these and the reference colony in the two study years. As a strict piscivore, the double-crested cormorant occupied a relatively higher trophic level, which may have contributed to differences in burdens of TEQs between species while concentrations of most other contaminants were largely similar between species.

Determinations of total clutch volume and intraclutch variation in 3-egg clutches can be used as an indicator of potential food stress for laying females during the egg production period. Total clutch volume did not differ significantly among the three herring gull colonies in all three study years indicating that food availability was likely not limited during this critical time. While mean intraclutch variation in egg size was statistically comparable among colonies in two of three years, mean intraclutch variation in egg size was significantly higher at Welcome Islands-a compared to Double Island in 2012. This variation in egg size at Welcome Islands-a however did not appear to impact chick survival, i.e., productivity values were normal. In 2001 and 2002, similar measurements of 3-egg clutches at Mutton Island were taken to determine total clutch volume and intraclutch variation in egg size (Supplementary Information, Table S5 and Table S6; ECCC unpublished). Egg size parameters at Mutton Island were comparable between 2001 and 2002 and a decade later in 2012, 2014 and 2015. Over a relatively longer time period from 1981 to 2014, significant declines in egg volume were found in herring gulls from colonies on the upper Great Lakes including those on Lake Superior (Hebert *et al.* in prep.). This finding has been associated with temporal changes in gull diet that may be related to reductions in prey fish availability.

Three additional endpoints relating to growth, development, and immune function of chicks were also measured in this study. Corticosterone deposited in growing feathers provides important insight into the physiology of stress during the period of feather growth (Bortolotti *et al.* 2009). Unexpectedly, feathers from gull chicks at the reference colony had significantly higher mean concentrations of corticosterone compared to chicks from AOC colonies in two of three study years. Possible stressors at the reference colony could include low food availability, inclement weather, and/or threats of predation. Depressed thyroxine concentrations in reference colony chicks (albeit significant in 2012 only) could have implications in terms of body condition and growth and warrants further investigation. Immune function assessed using the PHA skin response test was suppressed in herring gull chicks that had high levels of PCBs, 2,3,7,8-TCDD, and total TEQs (Grasman *et al.* 2013). In this study, there was no evidence of suppressed immune function in chicks from AOC colonies in 2015. Results for all three of these endpoints suggest that no adverse health effects were found in AOC chicks compared to reference colony chicks. Early studies examining corticosterone in plasma of gull chicks from Welcome Islands-b in 2000 and 2008 indicated no significant differences in stress response in gulls from the AOC colony compared to the reference colony (ECCC unpublished); further details of these studies are provided in the Supplementary Information, Figure S1.

In conclusion, the results of this three-year study suggest that there is no evidence of impaired reproduction or deformities in herring gulls attributable to local contamination effects within the

Thunder Bay AOC. Embryonic viability of herring gulls was high at AOC colonies in 2012 and 2014 and productivity was sufficiently high to maintain a stable population in 2012, 2014 and 2015. No embryonic deformities were evident in gull eggs incubated in the laboratory and no morphological deformities were found in $\geq$ 21-day-old herring gull chicks from AOC colonies. Importantly, contaminant burdens (e.g., PCBs, 2,3,7,8-TCDD, and mercury) in gull embryos and double-crested cormorant eggs from the Thunder Bay AOC were not notably elevated and were comparable to or lower than burdens in embryos or eggs from respective reference colonies in these years. Population trends based on nest count surveys indicate greater numbers of nests for both species at AOC study colonies in 2007 and 2012 compared to earlier decades. Large declines in concentrations of sum PCBs and other organochlorines, 2,3,7,8-TCDD, and total TEQs in herring gull tissues since the early 1990s indicate that exposure to these compounds has decreased in herring gulls foraging in the AOC. While no change in mercury levels was found in herring gulls in the AOC, this temporal pattern was similar to that found at two other Lake Superior herring gull colonies during this period. The results of early studies conducted by CWS in the AOC in the 2000s support many of the findings presented in this current study. In summary, concentrations of PCBs, other organochlorine compounds, PBDEs, dioxins and furans, and mercury were not sufficiently elevated to adversely impact the reproductive success and development of herring gulls and cormorants nesting in the Thunder Bay AOC.

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Supplementary Information

i) Temporal Trends of Contaminants in Eggs of Herring Gulls and Cormorants in/near the Thunder Bay AOC:

Table S1. Temporal trends for concentrations of organochlorine pesticides, PCB 1:1, sum PCBs, sum PBDEs (µg/g) and 2,3,7,8-TCDD and 2,3,7,8-TCDF (ρg/g, wet weights) in herring gull eggs (a) from Mutton Island in the Thunder Bay AOC in 1992, 2000, and 2007 (where data are available). Concentrations of organochlorines, 2,3,7,8-TCDD and 2,3,7,8-TCDF in eggs of double-crested cormorants (b) from Cone Island, just outside of the AOC boundary, in 1989 are also shown. Samples are based on analysis of a single pooled sample consisting of 9 or 10 eggs. "-" indicates that data are not available. Concentrations in gull embryos from Mutton Island (as a mean) and cormorant eggs from Welcome I.-a (as a single pool) in 2012 and 2014 are also shown for reference.

a) Herring gull eggs/embryos:

Year	Colony	*p,p'*-DDE	Sum Chlordane	HCB	Dieldrin	HE	Mirex	OCS	PCB 1:1	Sum PCBs*	Sum PBDEs**	2,3,7,8–TCDD	2,3,7,8–TCDF
1992	Mutton I.	3.815	0.324	0.040	0.157	0.075	0.039	0.0010	14.380	7.334	NA	8.20	2.5
2000	Mutton I.	1.417	0.106	0.011	0.031	0.032	0.036	0.0017	6.688	3.165	0.455	5.40	0.75
2007	Mutton I.***	0.659	0.052	0.009	0.020	0.012	0.080	0.0011	4.137	2.204	0.567	2.33	*I*
2012	Mutton I.	0.261	0.023	0.008	0.007	0.006	0.005	0.0007	NA	0.746	0.258	1.44	*I*
2014	Mutton I.	0.258	0.027	0.009	0.017	0.008	0.017	0.0013	2.730	1.068	0.689	2.27	*I*

b) Double-crested cormorant eggs:

Year	Colony	*p,p'*-DDE	Sum Chlordane	HCB	Dieldrin	HE	Mirex	OCS	PCB 1:1	Sum PCBs*	2,3,7,8–TCDD	2,3,7,8–TCDF
1989	Cone I.	3.531	0.158	0.013	0.143	0.043	0.026	0.004	8.953	4.043	12	2
2012	Welcome I.-a	1.337	0.010	0.006	0.024	0.007	0.003	0.001	NA	0.921	-	-
2014	Welcome I.-a	1.070	0.014	0.008	0.028	0.008	0.008	0.001	2.320	1.180	3.07	*I*

* Based on the sum concentration of 35-62 PCB congeners

** Based on the sum concentration of 14 or 15 PBDE congeners; NA denotes that data are not available

*** Based on collections of dead eggs from egg viability study

I = possible interference

Table S2. Mercury concentrations (µg/g, wet weight) in herring gull eggs from Mutton Island in the Thunder Bay AOC in 1992, 1993, 1996, 2000, and 2007. Results are based on analyses of a single pooled sample of 9-13 eggs with the exception of eggs from Mutton Island in 1993 and 1996 where two pools of five eggs were analyzed and mean concentrations (SD) are shown. Note that as a result of differences in chemical methodology, concentrations in eggs collected from 1992-1996 have been adjusted as described in Weseloh *et al.* (2011) to allow for comparisons to current data. Mean mercury concentrations (SD) in gull embryos from Mutton Island in 2012 and 2014 are also shown for reference.

Year	Colony	Mercury
1992	Mutton I.	0.140
1993	Mutton I.	0.081 (0.016)
1996	Mutton I.	0.138 (0.043)
2000	Mutton I.	0.150
2007	Mutton I.	0.111
2012	Mutton I.	0.086 (0.042)
2014	Mutton I.	0.054 (0.028)

ii) Egg Viability (2007)

Herring gull egg viability was determined at Mutton Island in 2007 as part of a broad study of biological effects associated with contaminant exposure in herring gulls from the Great Lakes from 2000-2007. Egg viability was determined using an embryonic viability detector (EVD) that detects vibrations associated with embryonic movement and heartbeat. These vibrations are turned into sound waves allowing for a determination of whether an embryo is dead or alive. Eggs were examined at 15-20 days of incubation and egg viability was calculated as the proportion of live eggs in a 3-egg clutch. Egg viability was also determined at Chantry Island on Lake Huron, which serves as the non-AOC Great Lakes reference site for comparison purposes. Herring gull egg viability at Mutton Island in the Thunder Bay AOC in 2007 was high and equal to 94.1% on average (Table S3). Egg viability was also high and equal to 96.8% at Chantry Island in 2000-2004. No significant difference in egg viability was found between the two colonies.

Table S3. Mean percentage (SD) of viable herring gull eggs in 3-egg clutches from Mutton Island in the Thunder Bay AOC and Chantry Island in Lake Huron as the reference colony in years shown and where data are available. N indicates the number of 3-egg clutches.

Colony	Year	% Viable	N
Mutton I.	2007	94.1 (12.8)	45
Chantry I.	2000-2004	96.8 (10.3)	229

iii) Populations of Nesting Colonial Waterbirds at Thunder Bay AOC Study Colonies and Lake Superior:

Table S4. Census data of herring gull and double-crested cormorant nests (=pairs) at study colonies in the Thunder Bay AOC (A) and for all colonial waterbirds on the Canadian and American sides of Lake Superior (B) during the 1st (1977/1978), 2nd (1989-1991), 3rd (1996-2001) and 4th (2007-2010) decadal surveys (Scharf 1978, 1998; Blokpoel *et al.* 1980; Blokpoel and Tessier 1993, 1998; Scharf and Shugart 1998; Cuthbert *et al.* 2001; CWS unpublished; Cuthbert and Wires unpublished). Decadal survey years in the Thunder Bay AOC are shown by an asterisk and nest counts for other years were collected during assorted field studies by CWS in the AOC. Percent change in nest numbers for colonial waterbirds in Lake Superior are between the 3rd to 4th decadal surveys.

A) Herring gulls – AOC study colonies:

	1978*	1989*	1999*	2000	2001	2002	2007*	2008	2012	2014	2015
Mutton Island	50	133	114	NC	182	196	180	177	171	142	129
Welcome Islands-a	0	70	164	0	NC	NC	255	NC	239	148	118
Welcome Islands-b	400	229	241	216	NC	NC	433	NC	347	NC	NC
Total	**450**	**432**	**519**	**-**	**-**	**-**	**868**	**-**	**757**	**-**	**-**

NC=not counted

Double-crested cormorants – AOC study colonies:

	1978*	1989*	1996*	2000	2007*
Welcome Islands-a	0	62	69	131	455
Welcome Islands-b	1	73	261	421	1,119
Total	**1**	**135**	**330**	**552**	**1,574**

B) Colonial waterbirds – Lake Superior:

Species	Census Year				Percent
	1977/78	1989-1991	1996-2001	2007-2010	Change
Herring Gull	13,269	24,896	18,987	15,255	-19.7%
Ring-billed Gull	7,471	15,742	18,761	15,656	-16.6%
Double-crested Cormorant	35	1,975	4,270	4,789	+12.2%
Great Blue Heron	527	685	466	343	-26.4%
Common Tern	328	282	316	315	-0.3%
American White Pelican	0	0	0	20	-
Caspian Tern	0	0	0	4	-
Black-crowned Night-Heron	0	0	0	3	-
Totals	**21,630**	**43,580**	**42,800**	**36,385**	

iv) Egg Size Parameters (2001 & 2002):

Significant spatial variation was found for total clutch volume in 3-egg clutches from Mutton Island compared to other colonies in both 2001 and 2002 (Table S5). In both years, mean clutch volume was significantly smaller in clutches from Mutton Island compared to the Chantry Island reference colony. In one of two years, total clutch volume was significantly smaller in clutches from Mutton Island compared to Granite Island, another gull colony on Lake Superior. Mean intraclutch variation (ICV) in egg size was not significantly different among the three colonies in both years. There was no evidence of temporal changes for either of these endpoints in 2001 and 2002 compared to current estimates in clutches of eggs in 2012, 2014, and 2015 (Table S6).

Table S5. Mean total clutch volume (SD) and intraclutch variation (SD) in 3-egg clutches of herring gulls from Mutton Island, Granite Island and Chantry Island in 2001 and 2002.

Colony	Year	Clutch Volume (cm^3)	ICV (%)	N
Mutton I.	2001	241.23 (19.43) **B**	9.48 (4.75)	152
Granite I.	2001	251.08 (18.05) **AB**	10.51 (6.35)	30
Chantry I.	2001	256.48 (16.87) **A**	8.23 (4.32)	63
Mutton I.	2002	240.51 (22.76) **B**	12.35 (13.91)	31
Granite I.	2002	257.17 (16.12) **A**	8.84 (5.02)	30
Chantry I.	2002	255.0 (14.23) **A**	9.63 (3.64)	48

Table S6. Temporal trends in mean total clutch volume (SD) and intraclutch variation (SD) in 3-egg clutches of herring gulls from Mutton Island in 2001, 2002, 2012, 2014, and 2015. N indicates the number of 3-egg clutches.

Year	Clutch Volume (cm^3)	ICV (%)	N
2001	241.23 (19.43)	9.48 (4.75)	152
2002	240.51 (22.76)	12.35 (13.91)	31
2012	250.96 (18.32)	10.66 (3.88)	12
2014	236.94 (9.83)	10.02 (7.66)	9
2015	243.68 (18.18)	8.64 (3.20)	12

v) Stress Response in Juvenile Herring Gulls in the Thunder Bay AOC (2000 & 2008):

In order to assess adrenal responsiveness, two different methods were employed to simulate stress in juvenile chicks from Welcome Islands-b in 2000 and 2008. In 2000, chicks underwent a standardized capture, handling and restraint procedure known to elicit an increase in circulating corticosterone levels. In 2008, chicks were injected with ACTH as a standardized stress challenge. Details for both methods are provided in Mayne *et al.* (2004). Following the stress event, blood samples were collected at regular intervals to measure the bird's ability to respond through the production of corticosterone. Juvenile chicks, i.e., between 21 and 28 days of age, were captured and housed separately prior to the stress test. Response was measured in eight chicks from Welcome Islands-b in 2000 and 12 chicks in 2008. In

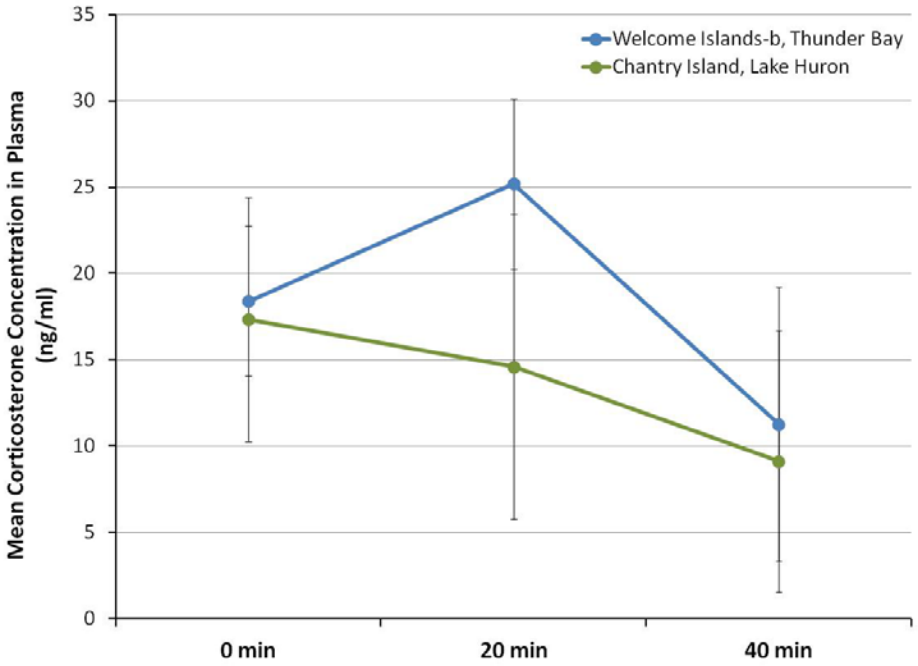
2000, where handling chicks represented the stress challenge, serial blood samples were taken at 0, 20, and 40 minutes. For the ACTH challenge, birds were injected with ACTH (Cortosyn, Amphastar Pharmaceuticals, 5 µg/kg body weight) at staggered times. Serial 0.5 ml blood samples were then taken from each individual at 10, 25, and 45 minutes post-injection. Hereafter, methods for quantifying corticosterone in plasma were similar in the two study years. Blood was transferred to a microcentrifuge tube containing 5 µl heparin and mixed by inversion and kept on ice until centrifugation. The separated plasma and red blood cells were then frozen in liquid nitrogen. The plasma samples were analyzed for corticosterone by radioimmunoassay (IMMUNOCHEM double antibody corticosterone ^{125}I RIA kit [for rats and mice]; ICN #07-120103, Santa Ana, CA, USA) as per the kit instructions. In this test, a limited amount of specific antibody is reacted with ^{125}I-labeled corticosterone. When an additional amount of corticosterone is available there is less ^{125}I-labelled corticosterone bound to the antibody. After separation of the bound from the free ^{125}I-labelled corticosterone, the amount of radioactivity is measured and the amount of hormone present in the sample can be determined from a standard curve. Statistical comparisons were made to juvenile chicks from Chantry Island on Lake Huron in 2000 (N=7 chicks) and 2003 (N=16 chicks) where these tests were also done.

In 2000, corticosterone response was not significantly different between juvenile chicks from the Thunder Bay AOC colony and the Chantry Island reference colony following the handling challenge (repeated measures ANOVA; Figure S1a). As expected, there was a significant difference in the secretion of corticosterone over the three time periods following the handling challenge (time effect; $p=0.0003$). No significant difference was found in the pattern of corticosterone secretion between the two colonies (site x time effect).

In 2008, a similar pattern was found following the ACTH challenge (Figure S1b). Corticosterone response was not significantly different between juvenile chicks from the Thunder Bay AOC colony and the Chantry Island reference colony or the pattern of secretion between the two colonies (repeated measures ANOVA). There was a significant difference in the secretion of corticosterone over the three time periods following the ACTH challenge ($p=0.000001$).

Figure S1. Mean (SD) corticosterone levels in plasma of juvenile herring gulls from the Thunder Bay AOC (Welcome Islands-b) with no ACTH challenge (a) and following an ACTH challenge (b) in 2000 and 2008 (N=8 and 12 chicks, respectively). Chantry Island on Lake Huron served as the reference colony in both studies where chicks were sampled in 2000 and 2003 (N=7 and 16 chicks, respectively).

a)



b)